ALDOSTERONE IN CLINICAL AND EXPERIMENTAL MEDICINE

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BLACKWELL
SCIENTIFIC PUBLICATIONS
OXFORD

East Lawrence Avenue, Springfield, Illinois
Published simultaneously in Canada by the Ryerson Press, Queen Street West, Toronto 2.

FIRST PRINTED 1959

PRINTED IN GREAT BRITAIN IN THE CITY OF OXFORD
AT THE ALDEN PRESS
BOUND BY THE KEMP HALL BINDERY
OXFORD

Preface

Aldosterone was discovered only 5 years ago and its study is still bedevilled by methodological difficulties. Its concentration in the blood is too low to be measured by methods routinely available at the present time. Some 5%, at best, of the amount of aldosterone secreted by the adrenal cortex in a day is recoverable in the urine and yet our knowledge of the production of the hormone in man must perforce be derived from figures obtained from the analysis of urine. Methods which may enable an estimate to be made of the amount actually secreted by the adrenal cortex are still in their infancy. An additional serious difficulty is the lack of a specific and reliable method for the estimation of the small quantities that do appear in the urine.

Despite these difficulties of approach, an enormous amount of work has been done, much of it of necessity by crude methods, and a whole structure of 'facts' and hypotheses has been erected on these rather tenuous foundations concerning the secretion, control and significance of aldosterone in health and disease.

Until recently, difficulties of synthesis have prevented more than minute amounts of the hormone being available for the investigation of [illegible] beginning to emerge. [illegible]

This monograph represents an expansion of material submitted for the degree of Doctor of Medicine of the University of London. It attempts to survey the data at present available concerning the subject and, in the final chapter, to present an appraisal of its significance in homeostasis.

[illegible] Thorn, Luis Hernando, Jean [illegible] Don Nelson and Albert [illegible] the Peter Bent Brigham Hospital, for stimulating his interest in this subject, and to the Medical Research Council for making possible the visit to Boston. He is also grateful to Professor M. L. Rosenheim for the unenviable task of reading the manuscript, and to Miss E Tinlin for the more unenviable task of typing it.

Contents

Nomenclature

Aldosterone (electrocortin)	11β:21-dihydroxy, 3:20-dioxo-pregn-4-ane, 18-al
Corticosterone (compound B)	11β:21-dihydroxy-pregn-4-one, 3:20-dione
11-deoxycorticosterone (deoxycortone) (cortexone)	21-hydroxy-pregn-4-ene, 3:20-dione
17-hydroxy, 11-deoxy-corticosterone (substance S)	17α:21-dihydroxy-pregn-4-ene, 3:20-dione
11-dehydrocorticosterone (compound A)	21-hydroxy-pregn-4-ene, 3:11:20-trione
17-hydroxy, 11-dehydro-corticosterone (cortisone) (compound E)	17α:21-dihydroxy-pregn-4-ene, 3:11:20-trione
17-hydroxy-corticosterone (hydrocortisone) (cortisol) (compound F)	11β:17α:21-trihydroxy-pregn-4-ene, 3:20-dione
vasopressin ('pitressin') (adiuretin) (anti-diuretic hormone)	β-hypophamine

INTRODUCTION

The fatal consequence of the pathological destruction of the adrenal glands in man,[8] or of their experimental removal in animals,[44] was established 100 years ago. It was not until 1927 that the fall in plasma sodium and rise in plasma potassium concentrations which follow bilateral adrenalectomy in animals were observed[34] and attention thereby drawn to the role played in sodium metabolism by the secretions of the adrenal glands. This was followed by recognition that the symptoms of adrenal insufficiency in animals could be ameliorated by the administration of sodium chloride.[31, 76, 314, 305, 437] Identical observations were later made on patients suffering from Addison's disease and the protective effect of a high intake of sodium chloride in such patients appreciated. [272, 273] Adequate confirmation of these fundamental findings appeared within the next few years[6, 166, 197, 300, 330, 276, 416, 462, 470] and the beneficial effect of sodium chloride in the treatment of Addison's disease was established.[197, 334, 272, 274]

Attempts were naturally made to isolate the life-saving constituent elaborated by the adrenal gland. As far back as 1896, Sir William Osler had reported striking clinical improvement in a patient suffering from Addison's disease when given a glycerine extract of fresh hog adrenals by mouth.[349] It was only after the failure of the then newly synthesized medullary hormone, adrenaline, to maintain life in the adrenalectomized animal[322, 330] that it was recognized that the vital constituent originated in the cortex of the gland. In 1927 three groups of workers[174, 175, 207, 208] independently reported the preparation of extracts of the adrenal cortex which were claimed to prolong the survival of adrenalectomized dogs and cats. Little information is available about Goldzieher's[174, 175] preparation. [illegible]

[illegible]

The preparation of Hartman, MacArthur and Hartman[207] doubled the survival time of adrenalectomized cats and an ether extract later prepared by Hartman and Brownell[208] kept adrenalectomized cats alive 'indefinitely'[201] and was successful in rescuing a patient with Addison's disease from crisis,[206] as was the aqueous extract of Swingle and Pfiffner. [476, 477, 478] The acetone-ethylenedichloride method of extraction

developed by Cartland and Kuizenga[41] was adopted for the commercial preparation of lipo-adrenal extract from hog adrenals. Aqueous extracts of beef adrenals were also prepared commercially for clinical use. These early adrenocortical extracts were very expensive; they were not free from toxic contaminants, they were variable in their pharmacological properties and some batches were apparently entirely lacking in adrenocortical activity.[105]

Analyses of concentrates of whole adrenal glands revealed that these contained a large number of steroid substances which either originated in the adrenal cortex or were artefacts formed during the chemical manipulations. The first steroid to be isolated which possessed recognized adrenocortical activity was corticosterone, in 1937.[181] Cortisone had been isolated in the previous year but its biological activity had not been recognized at the time.[314, 394, 555] By 1943, the number of steroids isolated from adrenal extracts had grown to 28. The following nine compounds were shown to possess significant biological activity: corticosterone (Compound B of Kendall); 11-dehydrocorticosterone (Compound A of Wintersteiner and Pfiffner); 17α-hydroxy, 11-dehydrocorticosterone (cortisone, Compound E of Kendall); 17α-hydroxy, 11-deoxycorticosterone (Substance S of Reichstein); 17α-hydroxycorticosterone (hydrocortisone, cortisol, Compound F of Kendall); adrenosterone, oestrone and progesterone Of these, the most important quantitatively were cortisol, corticosterone and cortisone.[111, 191, 550] One analysis, as an example, showed the following composition for hog adrenal homogenate:[529]

corticosterone	9.05 mg	per kg	of adrenal gland		
cortisol	7 08 ,,	,,	,,	,,	
cortisone	3 56 ,,	,,	,,	,,	

Only minute amounts of 11-deoxycorticosterone were found in adrenocortical extracts, one analysis gives the figure of 15 mg. in 1000 kg. of adrenal tissue.[399, 401]

When all the then known crystalline steroids had been removed from adrenocortical extract there remained an 'amorphous fraction' which possessed great physiological activity as measured by its sodium-retaining effect on the kidney of the adrenalectomized dog,[199] by the survival test in rats[510] and by the Everse-de Fremery test.[134] In particular, the amorphous fraction contained at least 50% of the sodium-retaining activity of the original extract

Many attempts were made to isolate further compounds from the amorphous fraction Grollman[199] claimed that he had isolated a crystalline

material which was a hundred times as active in sodium-retaining capacity as was 11-deoxycorticosterone, but he was unable to identify the chemical nature of his material. A concentrate possessing high sodium-retaining activity in the adrenalectomized dog was also prepared by Hartman and Spoor.[illegible] This fraction could maintain an adrenalectomized dog in good condition for long periods despite a low plasma sodium concentration. The substance responsible for this effect on sodium metabolism was named the 'sodium factor'.[illegible] This factor posesssed considerably more sodium-retaining activity than 11-deoxycorticosterone on a weight basis. It does not appear to have been identical with aldosterone since it was insoluble in chloroform and in ethyl alcohol.

The enterprise of the organic chemist, particularly of groups in Basel and Princeton, at the Mayo Clinic and Columbia University led to the availability of individual corticosteroids for the investigation of their physiological and pharmacological actions. Those particularly active in the field of carbohydrate metabolism were characterized as 'glucocorticoids', those with strong sodium-retaining activity as 'mineralocorticoids'.[illegible]

Steroids which were active in carbohydrate metabolism were known to be secreted by the adrenal cortex in amounts adequate to explain the physiological activity of the gland. The activity of the adrenal cortex in the metabolism of sodium and potassium had not been satisfactorily accounted for by the compounds then known. It was becoming recognized that the rigid classification of corticosteroids into those with purely 'glucocorticoid', and those with purely 'mineralocorticoid', activity was unrealistic, since these hormones have activities with respect to both carbohydrate and mineral metabolism, although differing in degree in these two spheres. Some investigators[illegible] [illegible] suggested that it was unnecessary to postulate the existence of a separate hormone possessing especially potent activity on sodium and potassium metabolism. Others believed that the hormone responsible for 'mineralocorticoid' activity was yet to be discovered.

From 1950 onwards, suggestions that such a hormone did in fact exist began to appear from various sources. Just as elucidation of the activity of corticosteroids in the sphere of carbohydrate metabolism had been advanced by the development of reliable methods of assay, so the final isolation of aldosterone was greatly facilitated by the development of a simple, reliable method of bioassay. This was based on its effect on the excretion of sodium and potassium in suitably prepared adrenalectomized rats.[illegible]

Using this method of bioassay, Tait, Simpson and Grundy[illegible], [illegible]

compared the activity, weight for weight, of beef adrenocortical extract with that of the known crystalline steroids that had been isolated from adrenocortical extract. In their assay, only two compounds, 11-deoxycorticosterone and 11-deoxycorticosterone 21-acetate, were more potent than adrenocortical extract. An attempt was then made to isolate the compound responsible for the sodium-retaining activity of adrenal extract. Using a chromatographic method of separation, namely the toluene-propyleneglycol system of Burton, Zaffaroni and Keutman,[12] 87% of the original activity of the adrenocortical extract was found to run at the same rate as the cortisone reference standard. The active substance could not have been cortisone, however, since the mineralocorticoid activity of the cortisone band was much greater than that of the actual amount of cortisone present in the extract, as determined chemically. It was therefore concluded that the sodium-retaining activity was due to an unknown substance with running properties, in the chromatographic system used, which were identical with those of cortisone. In this chromatographic system, the two known hormones with potent sodium-retaining activity, namely 11-deoxycorticosterone and 17-hydroxy, 11-deoxycorticosterone, ran off the paper

Simpson, Tait and colleagues next proceeded to extract and fractionate by chromatographic methods the steroids from adrenal venous blood of a rhesus monkey and a dog[441] The cortisone spot on the chromatogram from the extract of monkey blood, which contained only 1-2 µg of cortisone, possessed an activity in their bioassay equivalent to 15 µg. of 11-deoxycorticosterone, that is, seventy-five times the expected activity of the cortisone actually present. In the extract from the dog's blood, the effect on the sodium/potassium ratio of adrenalectomized rats was fifty-six times that of the amount of cortisone isolated from the spot on the chromatogram.

It was obvious from these results that there was present in adrenal gland extract and in adrenal venous blood a hormone more potent in its action on sodium and potassium metabolism than any hitherto described. This substance was provisionally termed 'electrocortin'.[444] Separation of this compound from cortisone was achieved by using a second chromatographic system, the benzene-aqueous methanol system of Bush.[68] By this means about 20 µg. of a reasonably pure preparation were made.[442] This material was found to have an absorption peak in ultra-violet light at 238 mµ. A larger sample was then prepared by fractionation on kieselguhr partition columns[443] of adrenocortical extract derived from 60 kg. of beef adrenal glands. Biological activity, determined by alteration of the sodium/potassium ratio in the urine of adrenalectomized rats,

was found to reside in the fraction which had an ultra-violet absorption peak at 238 mμ, and which gave positive reactions in the blue tetrazolium[illegible] and soda fluorescence[illegible] tests. It also corresponded with a spot seen on the benzene-aqueous methanol paper chromatogram with a running value between cortisol and cortisone; on the toluene-propyleneglycol system of Zaffaroni[illegible] it ran a little faster than cortisone. The substance concerned ('electrocortin') when acetylated with ^{14}C-acetic anhydride ran as a single substance on the benzene-formamide system of Zaffaroni[illegible] with maximum radioactivity which lay between 11-deoxycorticosterone acetate and 17-hydroxy, 11-deoxycorticosterone acetate used as reference standards. The acetylated compound had no biological activity with respect to sodium retention, but activity was regenerated when the acetate was hydrolysed.[illegible] -

The final stage in the isolation of the pure substance was the result of a collaborative effort between groups of workers at the Middlesex Hospital, London, the Research Laboratories of Ciba Ltd., Basel, and the Department of Organic Chemistry of the University of Basel (Simpson, Tait, Wettstein, Neher, v. Euw, Schindler and Reichstein[illegible]). Large-scale column chromatography of beef adrenocortical extract prepared according to the method of Cartland and Kuizenga[illegible] was employed using kieselguhr columns with water as the stationary phase and petroleum ether, benzene and chloroform as the mobile phase. The 'electrocortin' fraction from this column was then run in the B2 column chromatogram of Bush.[illegible] Crystalline 'electrocortin' was isolated from the eluates of this column. The yield was 40 to 95 μg per kilogram of fresh beef adrenal gland. A similar crystalline material with strong sodium-retaining properties was obtained shortly afterwards by Mattox, Mason and Albert[illegible] and by Knauff, Nielson and Haines.[illegible] Mattox and Mason[illegible] later obtained a yield of 48.3 μg. of aldosterone per kilogram from beef adrenal gland.

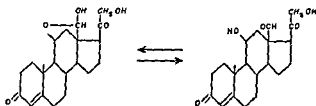
The constitution of the new steroid was elucidated in 1954 by Reichstein and colleagues[illegible] and its chemical properties described. It is 18-oxycorticosterone or corticosterone-18-aldehyde and has accordingly been given the definitive name of 'aldosterone'. In solution, this aldehyde form exists in equilibrium with the cyclohemiacetal form, the cyclohemiacetal predominating (Fig. 1). This chemical structure has been confirmed.[illegible] It will be seen that the compound does not possess a 17-hydroxy group and hence does not give the Porter-Silber reaction. It possesses an α-ketol side-chain and hence reacts with blue tetrazolium and it is a delta-4,3-αβ-unsaturated ketone and so gives a yellow soda fluorescence with ultra-violet light. Unlike other corticosteroids which

give only one acetate in the twenty-one positions, aldosterone can be acetylated to give three compounds, the 18- and 21-monoacetates and the 18,21-diacetate. These reactions are useful in the detection and estimation of aldosterone.

The total synthesis of DL-aldosterone has been achieved chemically by Schmidlin, Anner, Billeter and Wettstein[451, 512a]. The racemic compound has been resolved by microbiological methods into the biologically active D-isomer by Vischer, Schmidlin and Wettstein.[515a]

While this successful isolation of aldosterone from the adrenal cortex [illegible]

these chains of evidence was initiated by Grollman and Firor,[150] who found that extracts of human urine prolonged the life of adrenalectomized



ALDOSTERONE

Fig 1 Structural formula of aldosterone

cats; it was brought to fruition by Luetscher and collaborators, in a long series of papers from San Francisco Using sodium retention in the adrenalectomized rat preloaded with saline as a method of assay, and deoxycorticosterone acetate as reference standard, Deming and Luetscher[102] found that sodium-retaining activity was present in chloroform extracts of acidified urine obtained from patients suffering from disorders characterized by oedema, that is, in congestive heart failure, cirrhosis of the liver and in the nephrotic syndrome. Unfortunately, the sodium intake of these *patients at the time of their urine collections is not stated.* Furthermore, the urine extracts were not chromatographed before injection into the rats; the authors affirm, however, that the cortisol and cortisone present in the crude chloroform extracts were insufficient in amount for their action on the renal tubule to interfere with the sodium-retaining effect of the 'sodium-retaining factor'.

The elevated levels of sodium-retaining factor excreted in these patients'

urines were noted to decline when a diuresis occurred spontaneously[illegible] or was induced by the administration of cortisone or corticotrophin.[illegible] The observation of increased amounts of sodium-retaining factor in chloroform extracts of acidified urine from patients with nephrosis was also made by Singer and Venning[illegible] and by McCall and Singer.[illegible] The [illegible]

acetate) and greatly increased activity in the urine of a patient with cirrhosis. Normal subjects placed on dietary restriction of sodium were found to excrete an increased quantity of sodium-retaining factor compared with the level when the sodium intake was within the normal range.[illegible] More surprisingly, normal values were found in the urine of patients with hypopituitarism,[illegible] in contrast to patients with Addison's disease where sodium-retaining activity was not detectable in the urine.[illegible]

Later workers have amply confirmed the presence of a sodium-retaining factor in the urine of normal subjects, and of elevated amounts in the urine of cases of congestive cardiac failure, cirrhosis and nephrosis.

Chromatography of the chloroform extract of acidified urine by the toluene-propyleneglycol system of Zaffaroni, Burton and Keutman[illegible] showed that the substance responsible for the sodium-retaining activity of urine extracts had running properties equal to those of cortisone but could not be cortisone since this steroid had no sodium-retaining activity in the bioassay used.[illegible] Further chromatographic separation of the material eluted from the cortisone strip on the Zaffaroni chromatogram has proved that the 'sodium-retaining factor' isolated from the urine of patients with the nephrotic syndrome[illegible] and cirrhosis[illegible] is identical with aldosterone.

Aldosterone has also been detected in blood. An indication that a potent sodium-retaining factor was present in adrenal vein blood was provided by Spencer in 1950.[illegible] He found sodium-retaining activity equivalent to 4 µg of deoxycorticosterone acetate per millilitre of serum, as assayed by its effect on the sodium excretion of adrenalectomized mice, in dog adrenal vein blood. No activity was detected in blood from the carotid artery of the same animal.

Sodium-retaining activity, later proved to be due to aldosterone, was found by Simpson, Tait and Bush[illegible] in adrenal vein blood from a dog and in blood perfused through the isolated adrenal gland of a monkey. Aldosterone [illegible]

Hirschmann [144]

In adrenal vein blood of rats, Singer and Stack-Dunne[441] obtained chromatographic evidence of the presence of aldosterone and corticosterone; no other steroid could be detected. More recently, Singer has isolated aldosterone from adrenal vein blood of nephrotic rats [444]

In man, minute amounts of aldosterone have been detected in peripheral venous blood[17, 440] (see p. 39).

CHAPTER II

METHODS AVAILABLE FOR THE DETERMINATION OF ALDOSTERONE IN BIOLOGICAL FLUIDS

AN adequate method of estimation of the concentration of aldosterone in body fluids and, ideally, of its rate of secretion by the adrenal gland, is a prerequisite for the assessment of the role played by the newly discovered hormone in health and disease.

At the present time, methods for the measurement of the secretion rate are still being explored[17, 174, 400] and are only available in a very few research centres. Our knowledge of the quantitative aspects of aldosterone secretion in man and in experimental animals has been derived almost entirely from measurement of the excretion of the steroid in the urine. Even for this purpose there is no method of estimation which is wholly acceptable. Reliance upon the determination of the amount of aldosterone extractable from urine as a measure of the quantity of this steroid actually secreted by the adrenal gland implies the assumption that the two are directly related, that is, that the compound is handled by the individual with regard to rate of degradation and clearance by the kidney in a constant manner both from day to day and under varying physiological conditions. Such an assumption is almost certainly not warranted.

✓The concentration of aldosterone in the urine is approximately one in 200 million, and in the blood one in 1000 million. Chemical methods which are both sufficiently sensitive and specific to detect the presence of such minute concentrations in biological fluids are not currently available. Alternatively, the estimation of aldosterone involves its complete isolation from all other compounds giving the same chemical reactions The instability of steroids precludes the use of the more simple chemical methods of separation and necessitates the employment of complex and clumsy physicochemical methods. It was therefore fortunate for the early workers in this subject that aldosterone possesses very potent specific biological properties which can be utilized to develop bioassay techniques capable of detecting and estimating, in a roughly quantitative manner, the amount of this steroid in biological fluids. It was due to the skilled use of both physicochemical and bioassay techniques that aldosterone was first discovered. ✓

The most urgent requirement in this field is for a simple, specific and accurate chemical or physicochemical method for the estimation of aldosterone The volume of investigative work that has been performed with

the currently available methods is remarkable, but much of this has been done with methods which are far from specific, reproducible or accurate and will require repetition when reliable methods become available.

Biological Assay

For purposes of bioassay, advantage is taken of the potent effects of this steroid on the renal tubular reabsorption of sodium and potassium and the absence of such effects by other naturally occurring adrenocortical steroids when administered in amounts normally found in biological fluids. The evolution of the methods used for bioassay will be briefly reviewed.

The activity of adrenocortical extracts and hormones with respect to electrolyte metabolism was originally assessed by their effect on the renal excretion of sodium and potassium in the adrenalectomized dog.[104] The expense of these dogs, and the difficulty of keeping them alive, were grave disadvantages of this method. In 1947 Dorfman, Potts and Feil[116] introduced the use of adrenalectomized rats, thus improving the sensitivity of the method and making it more economical. In this assay, urine was collected for 4 or 6 hours after the subcutaneous administration of the test substance in corn oil. It was found that the sensitivity of the method could be further increased by the use of a low sodium load. Increased accuracy of measurement of urinary sodium was obtained by using radioactive sodium. With this assay as little as 0.98 μg. of deoxycorticosterone acetate could be detected; a dose-response curve, however, was not given. The principles of this assay have become the basis of most subsequent bioassay techniques.

Spencer[221] in 1950 employed mice weighing 35 g., preloaded with saline, and by the use of a 3-point assay was able to detect 0.5 μg of deoxycorticosterone acetate from the degree of sodium retention produced. Insufficient data is given to permit the calculation of the index of precision.*

In their extensive investigation of the sodium-retaining factor of urine, Luetscher and co-workers[227] have used a method of assay based on that of Dorfman, but have taken advantage of the introduction of flame photometry for the more accurate measurement of sodium and potassium and have also introduced the use of ethanol as the solvent for the material extracted from urine by chloroform after hydrolysis at pH 1. They found that preloading with distilled water, rather than with normal saline, increased the sensitivity of the assay. With this technique a dose of 25 μg.

* The index of precision of the assay, λ is derived from the equation λ = S/b, where S = standard deviation of the response and b = the slope of the assay. The smaller the value of lambda (λ), the greater the precision of the assay.[113]

TABLE I

BIOLOGICAL ASSAY OF ALDOSTERONE IN URINE

Authors	*Hydrolysis*	*Extraction*	*Preliminary chromatography*	*Bioassay technique*	*Index of precision*	*Normal range of excretion in man (μg/24 hr)*
Kagawa *et al* [232]	H_2SO_4 to pH 1 overnight	Chloroform	Toluene-propyleneglycol	Sodium excretion in adrenalectomized rats		1.5-3
Johnson[226]	HCl to pH 1 24 hr	Chloroform	1. Toluene-propyleneglycol 2. Occasionally Bush 'C'	log K/Na in urine of adrenalectomized rats		1-3
Singer and Venning [449] modified[603]	HCl to pH 1 5 24 hr then β-glucuronidase after 24 hr.	Immediate with chloroform and	Bush 'B5'	Sodium excretion in adrenalectomized rats	0 24	0 8-9 5 (males)* 1.7-5 5 (females)†
Liddle *et al* [244]	HCl to pH 1	Continuous with methylenechloride	None	'aldosteroid index' in adrenalectomized dogs	0 24	Not stated
Laragh *et al* [252]	HCl to pH 1	Continuous with methylenechloride	None	Sodium excretion in adrenalectomized rats	0 23	1-4

* Mean and S D = 3 2 ± 1 6 (based on fifty determinations in twelve individuals).
† Mean and S D = 1.7 ± 1 4 (based on twelve determinations in five individuals).

of deoxycorticosterone produced maximum sodium retention. No index of precision is given. They noted that extracts of urine from non-oedematous patients showed some sodium-retaining activity but that the urine of patients with congestive cardiac failure and the nephrotic syndrome gave values well above the normal range. This was the first indication of the presence of a sodium-retaining factor in urine. This method of assay was later modified by Johnson[118] who made it a cross-over test, using nine rats for each assay, divided into three groups of three rats each, each group receiving in turn the solvent only, standard and unknown in a different order on 3 successive days. This procedure minimizes the variability between groups. The use of the logarithm of the change of the potassium/sodium ratio was found to be double the precision of the method, compared with the use of alteration in sodium excretion alone.

Marcus, Romanov and Pincus[213] also devised an assay based on that of Dorfman[114] and again substituted flame photometry for the measurement of sodium and potassium in place of the use of isotopes. Using rats preloaded with saline, they found that the change in sodium excretion in response to deoxycorticosterone acetate is much more sensitive than change in potassium excretion and that following the injection of this steroid there was a direct relationship between the percentage decrease in sodium excretion and the percentage decrease in urine volume. Of the various other substances tested (Compounds A, B, E, F, pregnenalone, and lipo-adrenal extract) only lipo-adrenal extract caused sodium retention; pregnenalone excepted, all caused increased potassium excretion.

The use of the ratio of excreted sodium to potassium (Na/K) was introduced by Simpson and Tait[427] in 1952 to improve the sensitivity of the assay. Using a double isotope technique whereby ^{24}Na and ^{42}K were injected simultaneously as a solution containing a low sodium and a high potassium load, the radioactivity due to each isotope in the urine was measured simultaneously by a method of differential counting. The Na/K ratio varied inversely with the logarithm of the injected dose of deoxycorticosterone acetate over a range of 0.8 to 4.0 μg. The assay was also improved by the use of 20% ethanol as the solvent for the test substance. Their procedure was to inject the substance subcutaneously, followed 1 hour later by the injection of the isotopes, and to collect urine for the next 2 hours. Using a balanced assay of six rats per group, the index of precision of this assay was 0.25 to 0.3. The advantage of the use of the ratio (Na/K) is that it reduces errors due to incomplete collection of urine during the test period, except in so far as the urine sample obtained may not be representative of the total period. These authors noted that adrenal cortical extract possessed very high activity in this assay, twice as great as

a saturated solution of deoxycorticosterone acetate in the same solvent, 'for a reason as yet unexplained'.

The assay developed by Singer and Venning[111] employed adrenalectomized rats with ligated urethrae given a load of 3.5 mg. of sodium chloride together with a tracer dose of ^{24}Na. Bladder urine was collected at the end of 5 hours and its radioactivity measured. The mean ^{24}Na excretion of the test group (six or nine animals) was then compared with that of a control group of the same size. A significant difference was obtained with 2 µg. of deoxycorticosterone acetate. A similar technique was developed by Kagawa, Shipley and Meyer[112] but in this case isotopic sodium was not used. Lobotsky, Hannye and Lloyd[171] found that the intravenous administration of the test substance through a polythene cannula in the jugular vein increased the sensitivity of this procedure.

The procedure used by Laragh and Stoerk[113] employs rats 24 hours after adrenalectomy, preloaded with saline. Three groups of three rats each are used and the total excretion of sodium of the group over a 5-hour period is measured. The degree of precision claimed is 0.23.

The adrenalectomized dog was selected by Liddle, Cornfield, Casper and Bartter[114] as the test animal because studies of renal function can be more easily performed in this species than in rats. They also chose the intravenous route for the administration of an ethanolic solution of crude extract of hydrolysed urine. Catheterized bladder urine was collected over a 4-hour period following the injection of the urine extract or of the deoxycorticosterone reference standard. Little change in sodium or potassium excretion was noted in the first hours; maximum change occurred [illegible]

excretion and derived an 'aldosteronoid index' (= 2.3 × potassium response minus sodium response expressed as mEq./min.). Using a 6-point cross-over procedure, this assay has an index of precision of 0.24. Although crude extracts of urine are used, interference due to the presence of cortisone and hydrocortisone (which cause sodium excretion in this assay) are not considered to be a source of error since large amounts (of the order of 1 mg. or more) are required to exert any effect

It is presupposed in these biological assays of crude extracts of urine that the whole of the activity of the extract in causing modification of the renal excretion of sodium and potassium is due to the presence of aldosterone This supposition is not warranted, but the specificity of the assay procedure can be improved by preliminary purification of urine extracts by paper or column chromatography. Thus in the original isolation of aldosterone, Tait, Simpson and Grundy[111] initially used one, and sub-

sequently two, paper chromatographic systems, before fractions eluted from the paper were submitted to bioassay. Luetscher and co-workers similarly employ preliminary chromatography in the toluene-propyleneglycol system of Burton, Zaffaroni and Keutman[81] and in a few instances follow this by the 'C' system of Bush.[82] Venning and co-workers use a single separation in the 'B5' system of Bush.[80,8] Farrell and co-workers[140, 141] in their estimation of aldosterone in adrenal vein blood employ separation on the toluene-propyleneglycol system followed by the hexane-propyleneglycol system on the acetylated eluate from the cortisone fraction of the first chromatogram.

Biological assays have many disadvantages. They are time-consuming and costly in animals. As a quantitative procedure, they are subject to the vagaries of biological variation and require the use of large numbers of animals to ensure a reliable assay. The result obtained merely indicates the range in which the potency of the test substance lies, derived from comparison with the effects of known amounts *not of aldosterone* but of deoxycorticosterone and confidence limits are rarely given. Nevertheless, bioassay was, until recently, the only method available for the detection of very small quantities of aldosterone.

Physicochemical Methods of Estimation

Since large volumes of fluid are required for the isolation of detectable quantities of aldosterone by physicochemical methods, the most practical source of body-fluid available for measurement is urine. In the interpretation of such measurements it must tacitly be assumed that the level of urinary excretion of the hormone reflects its level of secretion by the adrenal cortex

In the absence of a chemical reaction specific for aldosterone, it is necessary to isolate the steroid completely. The instability of aldosterone precludes the employment of the more common methods of separation used for organic compounds, as, for example, condensation with Girard's reagent and subsequent differential hydrolysis. Fortunately the separation of individual steroids from mixtures has been made possible by the development of systems of paper and column chromatography, in which advantage is taken of the differential partition of these compounds between the aqueous stationary phase on the paper and that of the organic solvents of the mobile phase. The various steps used in currently employed methods will be discussed in some detail

Paper chromatography of free steroids suffers from the disability that the running properties of these compounds relative to one another tend to remain more or less constant in the different systems employed. This

Table II

PHYSICOCHEMICAL METHODS OF ESTIMATION OF ALDOSTERONE IN URINE

Authors	*Hydrolysis*	*Extraction purification*	*Chromatographic systems*	*Final estimation*	*Normal range of excretion in man (µg/24 hr)*	*Remarks*
Neher and Wettstein [249]	HCl to pH 1 24 hr.	0.3 vol chloroform five times Silica gel column	1. Toluene-propyleneglycol 2. Bush 'C'	Visual comparison with hydrocortisone standards using blue tetrazolium and soda fluorescence on paper	0.3-3.0	Error ± 20% for range of 5-20 µg ± 40% *for range of 1-5 µg.*
Neher and Wettstein [250]	HCl to pH 1.5 24 hr.	0.2 vol. chloroform four times	1. Chloroform-formamide 2. Bush 'C'	As above	0.5-12.5	
Ayres, Garrod, Simpson and Tait [18]	HCl to pH 1 24 hr.	Continuous extraction with 0.5 vol. chloroform, 24 hr	1. Kieselguhr column Acetylation then 2. Kieselguhr column	Soda fluorescence	4.5-23.5	Radioactive hydrocortisone and aldosterone added to facilitate fraction-cutting and to enable estimate to be made of recovery
Nowaczynski *et al* [257]	HCl to pH 1 24 hr.	Continuous extraction with chloroform. Silica gel column	1. Ethyleneglycol-toluene 2. E_2B 3. Bush 'B5'	Ultra-violet absorption. Blue tetrazolium reaction.	2.2-10 (Mean = 5.1)	Numerous interfering contaminants detected and isolated
Hernando *et al* [218]	HCl to pH 1 48 hr	0.3 vol. chloroform twice Florisil column	1. Toluene-propyleneglycol 2. Bush 'C'	Blue tetrazolium reaction, later dinitrophenyl hydrazine in test tube	1-15 (Mean = 5.0 ± 3.0)	If visual estimate (UV photoprint) and DNPH values do not agree, further chromatography on E_2B system essential
Moolenaar [230]	HCl to pH 1 24 hr	0.1 vol. chloroform twice 0.2 vol. chloroform once toluene, light petroleum – 70% methanol partition	1. Toluene, N-octanol-methanol, water 2. Toluene, light petroleum – methanol, water	Dinitrophenyl hydrazine in test tube	5.5-13.0 males 2.5-8.0 females	

makes it difficult to separate aldosterone from contaminants with similar running properties. This difficulty can be overcome by introducing a transformation step, e.g. acetylation, between two chromatographic systems, the relative running properties of the acetylated compound being quite different from the free steroid alcohol.

HYDROLYSIS

The original isolation of aldosterone from adrenal extract by Tait, Simpson and Grundy[432] employed a single chromatographic separation in the toluene-propyleneglycol system of Burton, Zaffaroni and Keutman.[52] Sodium-retaining activity (as judged by the effect of the eluate from the paper in the bioassay procedure of Simpson and Tait[427]) resided in the fraction corresponding to the cortisone reference standard. Separation of the sodium-retaining factor from cortisone was achieved by running this fraction in a second chromatographic system, the benzene-aqueous methanol system of Bush.[53] This principle of successive paper or column chromatography in different systems became the basis of subsequent physicochemical methods of estimation of aldosterone.

The amount of aldosterone extracted from urine by this method was, however, only 0.5 μg./l. in a normal person on an ordinary diet. The quantity of free aldosterone extractable from urine was found to be greatly increased by hydrolysis with strong acid[378] at pH 1.0 or 1.5. Increased amounts were likewise found in urine hydrolysed with β-glucuronidase [434] The nature of the conjugates of aldosterone in the urine is not yet known; other corticosteroids are conjugated as glucuronides and sulphates. It would appear that aldosterone occurs in urine mainly as a conjugate which is not a glucuronide. The principal excretory product appears to be a 3-keto conjugate, which is split by hydrolysis at pH 1 to liberate the Δ^{4},3-ketone.[330b] Only 10%, or less, of the amount of aldosterone secreted by the adrenal gland is excreted in the urine in the free form after hydrolysis at pH 1.[325, 359] A proportion (about 10%) of secreted aldosterone is excreted in a reduced form, as tetrahydroaldosterone, conjugated with glucuronic acid.[430d]

The efficiency of hydrolysis by strong acids and of *enzymic* hydrolysis by β-glucuronidase have been compared by many workers.[12, 135, 305] *Considerably higher yield was obtained if the urine was allowed to stand* for 24 or 48 hours at pH 1 than if it were extracted immediately after acidification. Higher values were obtained after acid hydrolysis than after

Experimental investigation of the efficiency of hydrolysis by hydrochloric acid showed that hydrolysis at pH 1 or 1.5 for 24 hours releases less free aldosterone than when hydrolysis proceeds for 48 hours; still further amounts are released by more prolonged hydrolysis for 72 hours and 96 hours.

Beyond 48 or 72 hours the yield may, however, decrease with time [illegible] temperature. The yield, however, was very variable when six identical urines were subjected to this treatment.[11]

Hydrolysis with hydrochloric acid at pH 1 or 1.5 for 24 or 48 hours at room temperature has now been adopted in most methods of estimation as the initial procedure.

Extraction

The free (i.e. no longer conjugated) steroid is extracted from the urine by a suitable solvent. The efficiency of extraction depends on the partition coefficient of aldosterone between urine and solvent. Chloroform and methylene chloride are the solvents usually used, although to my knowledge the partition coefficient of aldosterone between urine and organic solvents has never been determined. It is assumed to be similar to the other corticosteroids. Chloroform has the disadvantage of forming an emulsion with urine unless the volume of chloroform is greater than the volume of urine, but the emulsion can be broken by centrifugation or prevented by saturation of the urine with ammonium sulphate. Some workers[115, illegible] use simple manual shaking of urine and chloroform in a separating funnel for a short period; others[illegible] claim a greater yield by using continuous extraction for 48 hours. Most workers now use continuous extraction with methylene chloride for 24 hours

The solvent pool obtained from the above procedure is washed with sodium carbonate or sodium hydroxide solution to neutrality. This removes a quantity of pigment and phenols which form water-soluble salts in an alkaline medium. The solvent is washed with distilled water and dehydrated by standing over anhydrous sodium sulphate

Chromatography

1 *Purification by adsorption on columns or partition between organic solvents*

Pigments and other contaminants which interfere with the running of paper chromatograms can be removed to a worthwhile extent by passing the urinary extract through a column of florisil (magnesium trisilicate[illegible])

or silica gel.[349] These substances adsorb corticosteroids which are subsequently eluted by washing the column through with 25% methanol in chloroform or 50% acetone in chloroform. Partition between aqueous methanol and toluene-ligroin or petroleum ether or between water and benzene can also be used for the removal of fat-soluble impurities.

2. *Paper chromatography*

In the toluene-propyleneglycol system[387] *initially* used by Tait, Simpson and Grundy,[451] aldosterone runs slightly faster than cortisone. In this system aldosterone is separated from cortisol, di- and tetra-hydrocortisone and di- and tetra-hydrocortisol. Separation of aldosterone from cortisone necessitates the employment of a system in which the running properties of these two compounds are not identical. The benzene-aqueous methanol system of Bush ('B5')[53] was found by Simpson and Tait[438] to give good separation of the two compounds. The *Bush* 'C' system (toluene-ethylacetate, aqueous methanol[53]) likewise gives satisfactory separation; in this system aldosterone runs with the cortisol reference standard. Acetylation of the eluate from the cortisone band of the toluene-propyleneglycol paper and subsequent running in the hexane-propyleneglycol system was used by Farrell[140] to effect separation of aldosterone from cortisone.

Simpson and Tait[441] used the 'C' system of Bush[52] followed by the toluene-propyleneglycol system[82] for the isolation of aldosterone from adrenal extracts and from adrenal vein blood. For the determination of aldosterone in urine, Neher and Wettstein[349] found that better separation was obtained by reversing the order and in their first published method used the toluene-propyleneglycol system[82] followed by the Bush 'C' system.[82] This reversed order has also been adopted in the modification of this method devised by Hernando, Crabbé, Ross, Reddy, Renold, Nelson and Thorn[215] Neher and Wettstein[350] later substituted a chloroform-formamide system for the propyleneglycol system. The chloroform-formamide system has the advantage of having a much quicker running time. The capacity of the chloroform-formamide system, however, is much more easily overloaded than is that of the toluene-propyleneglycol system, so that up to five papers may have to be used to run a single urine extract when the former system is used.[349] For this reason it is useful to retain both systems and use the one better-suited to the weight of urine extract obtained. The use of a toluene-ethylene glycol system has been suggested by Nowaczynski and Koiw,[355] it has the advantage of greater speed of development whilst retaining the sharp degree of resolution possessed by the toluene-propyleneglycol system.

Moonlenaar[illegible] employs a toluene-octanol-methanol-water system, followed by the 'B1' system of Bush.[illegible]

A method of estimation making use of the principles of isotope dilution has been devised by Ayres, Garrod, Simpson and Tait.[illegible] After acid hydrolysis at pH 1 for 24 hours and extraction with chloroform, separation is achieved by column chromatography on kieselguhr of the free steroids followed by acetylation of the appropriate fraction and further chromatography on a kieselguhr column. Labelled (^{14}C) diacetate of aldosterone is added after the acetylation process. The fraction of the eluate from the column containing maximum radioactivity is then chromatographed on paper using the 'B3' system of Bush[illegible] and an estimate made of the amount of aldosterone present by use of the blue tetrazolium reaction and soda fluorescence. The specific activity of the aldosterone is determined and from knowledge of the amount of radioactivity originally present, allowance can be made for loss during part, at least, of the procedure. A mean recovery of 77% is reported. Good correlation is claimed between the results obtained by this physicochemical method and those obtained by bioassay of the same urine using the procedure of Simpson and Tait.[illegible]

With the increasing availability of tritium-labelled aldosterone, methods of estimation by isotope dilution procedures can be expected to come into ✓ general use. Kliman and Peterson[illegible] claim that such a method is capable of detecting 0.01 µg. of aldosterone. If this is confirmed, the routine determination of circulating aldosterone in peripheral blood should become a possibility.

CONTAMINANTS

In an investigation of the purity of the aldosterone fraction obtained by running an extract of urine on a two-system method of separation, namely the toluene-propyleneglycol followed by Bush 'C', Nowaczynski, Steyermark, Koiw, Genest and Jones[illegible] found that in some instances the 'aldosterone' isolated was not a single entity, but could be separated into further compounds when run in other systems, e.g. the 'E_4B' or Bush 'B5'. In one instance when the eluate from the Bush 'C' paper was rechromatographed on the 'B5' system of Bush two ultra-violet absorbing compounds were found. One was genuine aldosterone, the other also gave positive reactions with blue tetrazolium and alkaline fluorescence. In other instances (patients with Cushing's syndrome) three further compounds were isolated from the 'aldosterone' fraction eluted from the Bush 'C' paper. None of these, however, gave a positive reaction with blue tetrazolium, although they all gave a positive alkaline fluorescence test. Whether these contaminants are all derivatives of aldosterone or not

is not yet clear. Their physicochemical properties are detailed in Nowaczynski's paper.[238] One of them, 'Compound III',[238] appears to be identical with a compound noted in urine by Gray and co-workers,[105] the origin of which is not in the adrenal gland, but in ingested citrus fruit or fruit juice.

A contaminant of aldosterone isolated by employing two paper chromatographic systems has also been reported by Hernando and co-workers.[215] In a few instances they noted that the 'aldosterone' separated on the Bush 'C' chromatogram also ran as a single spot on the Bush 'B5'[62] and chloroform-formamide[250] systems but separated into three spots when run on the 'E_2B'[125] system. Of these, one had running properties identical with pure aldosterone, the nature of the other two is unknown.

Nowaczynski, Koiw and Genest[238] emphasize that a minimum of three chromatographic systems are at present necessary to ensure the complete isolation of aldosterone. The systems used by Nowaczynski and co-workers are the toleune-propyleneglycol,[63] 'E_2B'[125] and Bush 'B5'.[62] A recovery rate of 83% is claimed for this procedure.

The employment of three paper chromatographic systems would therefore appear to be essential if the absolute purity of the material isolated from paper were to be assured. Most authors at present employ two chromatographic systems and assume the purity of their material if quantitative estimation by ultra-violet absorption, alkaline fluorescence and blue tetrazolium are in agreement. The employment of extra steps in procedure, of course, involves additional loss of material.

Final Quantitative Estimation

The quantity of aldosterone present in the urine extract after the final chromatographic separation may be determined by comparison with standards run simultaneously on the paper, or in a spectrophotometer after elution from the paper. Some workers[250] employ visual guesswork of the spot on the ultra-violet photoprint or of the colour developed by blue tetrazolium[264] or alkaline fluorescence on paper[64] using standards of cortisol for comparison. A fluorimeter for measuring alkaline fluorescence[63] on paper has been developed by Ayres and co-workers.[10] Elution of the aldosterone from paper and final estimation of the test tube by means of the blue tetrazolium reaction,[254] the diphenylhydrazine reaction[141] or potassium-butoxide fluorescence[1] is employed by others

The blue tetrazolium, and to a lesser extent the dinitrophenylhydrazine, methods, in addition to being non-specific, suffer from the disadvantage that the paper blanks are large and variable. A method of eliminating interfering substances from the final eluate obtained from the paper

chromatogram is given by Genest and co-workers.[illegible] The use of isonicotinic hydrazide has been suggested.[illegible] The paper blank is low, but the method is not very sensitive. The current method to which least exception can be taken appears to be the alkaline fluorescence on paper, developed by Ayres and colleagues.[illegible] It is capable of measuring 0.2 µg. with an error which is claimed to be 6%.

RECOVERY RATES

Venning and co-workers[illegible] obtained a recovery rate of 18% and Llaurado mentions 20%;[illegible] both used the bioassay procedure for the final evaluation. Hernando and co-workers[illegible] obtained a recovery of 20-30% by their physicochemical method. Mills,[illegible] in a single determination, obtained a recovery of 30% with a physicochemical method, but later[illegible] reported a recovery of 55%. On the other hand, Nowaczynski, Koiw and Genest,[illegible] employing three paper chromatographic systems, claim a recovery of 82%. Neher and Wettstein,[illegible] using a physicochemical method, were able to reach a recovery rate of 65 to 85%. Ayres, Garrod, Simpson and Tait[illegible] claim a recovery rate of 77% for their method of column chromatography. Their routine use of labelled (^{14}C) cortisol and aldosterone acetate in their procedure enables individual recovery rates to be calculated for each urine sample and the corresponding corrections made in calculating the amount of aldosterone present in the sample.

Physicochemical Properties of Aldosterone

The following are some of the physicochemical properties of aldosterone which have been used for its identification·

1 The ultra-violet absorption curve of free aldosterone in ethanol shows a peak at 238 mµ,[illegible] this is typical of Δ^4 and some other αβ-unsaturated ketones.

2 The infra-red spectrum of free aldosterone in chloroform shows a peak at 1696 cm.$^{-1}$ this is typical of a 20-carbonyl group in the ketol sidechain.[illegible]

3 The sulphuric-acid spectrum shows a peak at 288 mµ.[illegible]

4. The substance gives a bright yellow fluorescence with methanolic sodium hydroxide under exposure to ultra-violet light;[illegible] this is indicative of a Δ^4 3-ketone structure in ring A.

5 The substance gives an immediate blue tetrazolium reaction; the resulting formazan in ethanol shows a peak at 510 mµ This is indicative of an α-ketol side chain [210]

6. Aldosterone reduces ammonical silver nitrate; this is typical of α-ketols and α-ketoaldehydes.[82]
7. Aldosterone reacts with 2:4 dinitrophenylhydrazine to form hydrazones at each carbonyl group. The C_3 ketone reacts in 5 minutes to form a monohydrazone; after 90 minutes at 60°, a mixture of C_3, C_{18} and C_3 C_{18} dihydrazones are formed with maximum absorption at 460 mμ.[180]
8. Aldosterone yields formaldehyde on sodium-bismuthate oxidation; this is indicative of the presence of an α-ketol or glycol structure.[133]
9. Aldosterone does not react with phenylhydrazine in sulphuric acid (the Porter-Silber reaction) indicating that it is not a 17α, 21-dihydroxy, 20-ketosteroid.[133]
10. Aldosterone does not give the Steyermark-Nowaczyski reaction (heating in sulphuric acid previously dehydrated in phosphorus pentoxide) indicating that it is not a Δ^4—3, 20 dione, 17, 21 diol.[133]
11. Aldosterone has the following chromatographic characteristics:

Toluene-propyleneglycol[82]	*R_c = 1.05
Chloroform-formamide[150]	†R_f = 0.70
Bush B1[23]	R_f = 0 05
„ B5[23]	R_f = 0.30
„ C[23]	R_f = 0.40
E_2B[115]	R_f = 0.36
Toluene-octanol-aqueous methanol[130]	R_f = 0.40

* R_c—reference to cortisone. † R_f—reference to solvent front

Comment on Physicochemical Methods

The physicochemical methods at present available are cumbersome and time-consuming. It takes 8 days, for example, to process a single urine by the method of Hernando and collaborators.[111] Recoveries of added material appear to be poor and variable. Many workers do not quote figures for recoveries or for the reproducibility of their methods. However, the advent of labelled aldosterone will permit of greater accuracy of measurement by enabling allowance to be made for loss of material during processing in each individual sample. Methods available at present for the final quantitative estimation also leave much to be desired The field of aldosterone estimation impatiently awaits the discovery of a specific chemical method accurate to amounts of the order of tenths of a microgram.

ESTIMATION OF RATE OF SECRETION OF ALDOSTERONE BY THE ADRENAL CORTEX IN MAN

The preparation of aldosterone labelled with a radioactive tracer, *e.g.* tritium (^{3}H), has enabled the principles of isotope dilution to be used to obtain an estimate of the rate of secretion of aldosterone by the adrenal cortex in man.

Ayres and collaborators[14] injected [16-^{3}H]-aldosterone intravenously and measured the specific activity of the aldosterone excreted during the ensuing 24 hours. Calculations based on the specific activity of the injected and excreted material and the amount of aldosterone excreted in the urine during the 24-hour period gives the miscible pool of aldosterone. Assuming that there is no store of aldosterone in the body the miscible pool represents the amount of aldosterone secreted by the adrenal cortex during the collection period. The accuracy of this method will, of course, depend upon the accuracy with which urinary aldosterone and tritium can be measured.

Ulick and colleagues[11,14] have applied the isotope dilution method to a metabolite of aldosterone, tetrahydroaldosterone, which is excreted in the urine in amounts comprising 10 to 15% of the injected radioactivity. Following the injection of a known amount of tritiated aldosterone, the total amount and the specific activity of the tetrahydro metabolite excreted over the following 24 hours is determined. From these measurements the secretion rate can be calculated. It is assumed that aldosterone is the only precursor of this metabolite.

Since the injected radioactive material is presumably handled by the liver and kidney in exactly the same manner as the naturally secreted hormone, estimates of aldosterone secretion based on isotope dilution methods are not subject to the criticisms made against estimates based on the urinary excretion of aldosterone.

CHAPTER III

THE OCCURRENCE OF ALDOSTERONE IN BIOLOGICAL FLUIDS

A. Excretion of Aldosterone in the Urine

For the reasons outlined in the section on methodology, much more information is available concerning the quantity of aldosterone excreted in the urine than is known about its concentration in peripheral or adrenal venous blood or in other body fluids in man.

Imperfections in the methods used for the determination of aldosterone in the urine have resulted in many conflicting reports of the amount excreted in the various disease states surveyed in this chapter. The actual amounts found in the urine by different authors depends upon the method used for its estimation and will vary with the reproducibility of the method and the completeness of the recovery of free aldosterone. Less than 1% of the actual amount of aldosterone secreted by the adrenal cortex is recoverable as free aldosterone from the urine. Hydrolysis of the urine liberates aldosterone from conjugates, bringing the amount recoverable from urine up to about 5% of that secreted. The constancy of the percentage excreted in the urine will be altered by any variation or abnormality of the metabolism of aldosterone in the body and also by any alteration of the renal clearance of the hormone.

The hormone was of necessity estimated by the early investigation by methods involving bioassay, usually of crude extracts of urine. The results of these measurements are expressed in the text in terms of micrograms of 'sodium-retaining hormone' or 'sodium-retaining factor' or in deoxycorticosterone equivalents. Subsequently, many workers have continued to use bioassay techniques for the estimation of aldosterone in some cases (but by no means in all) after preliminary partial separation chromatographically from other biologically active substances which may antagonize or potentiate the biological activity of aldosterone. The results in these cases are quoted by the authors and in this text in terms of micrograms of aldosterone Positive chemical identification of the substance responsible for the biological effect as aldosterone has been done in very few instances.

It will be appreciated that values for aldosterone excretion should be accepted with reservation at the present time and the interpretation of results must be tentative.

The Normal Daily Excretion

The amount of aldosterone reported as the daily excretion in man varies with the method used for its estimation.

Reports of the daily excretion of sodium-retaining hormone (presumed aldosterone) as measured by bioassay have in general been lower than those found by physicochemical methods. Venning and co-workers found a mean of 3.2 µg. (S.D.±1.6) in fifty determinations on twelve normal men.[illegible] Laragh and Stoerk[illegible] give a value of 1 to 4 µg, and Axelrad and co-workers[illegible] report that the normal output is 2 to 4 µg. Genest and co-workers[illegible] found less than 1.4 µg./24 hr. in the urine of five adult males, but the urine was hydrolysed only by β-glucuronidase in their method. Liddle[illegible] obtained values of up to 12 µg /24 hr. by their bioassay method using intravenous injection into adrenalectomized dogs, as judged by Fig 7 of another paper from their laboratory.[illegible]

Thus measurement by bioassay, either without any chromatographic separation in some cases or after partial separation from other steroids in others, results in the majority of reports in values ranging from 1 to 3 or 4 µg /24 hr. Workers using physicochemical methods report higher figures (see Table I). Neher and Wettstein[illegible] give values for the daily excretion which range from 0.5 to 12.5 µg. The range found by the modification of Hernando and co-workers[illegible] in seventy-two determinations was 0.5 to 15 µg./24 hr. with a mean of 5.0±3.0 µg (S D.). Moolenaar[illegible] gives a range of 2.5 to 13 µg./24 hr. The range found by Ayres, Garrod, Simpson and Tait,[illegible] using a method of successive column chromatography and isotope dilution so that allowance is made for loss of material during the procedure, was 4.6 to 23.5 µg./24 hr. Values of 0.3 to 2.3 µg /48 hr. were found in babies during the first 2 days of life by Muller and Gautier,[illegible] using the method of Neher and Wettstein.[illegible] No significant sex difference has been noted.[illegible] No correlation has been noted between urine volume and aldosterone excretion.[illegible] This differs from the excretion of 17-hydroxycorticoids, which increases as urine volume increases.[illegible]

Individual daily variations have been followed in two normal subjects, not on a constant diet, for 15 and 20 consecutive days respectively.[illegible] In one case the excretion of aldosterone varied from 1 to 10 µg /24 hr.; in the other from 0 to 15 µg /24 hr. Some of this 'spontaneous' variation may be due to variations in the recovery of aldosterone by the method used.

Diurnal Variation

The observations of Luetscher,[illegible] Venning,[illegible] Muller[illegible] and Hernando[illegible] and their collaborators show that a diurnal rhythm of

urinary excretion of aldosterone, and so presumably of secretion by the adrenal cortex, occurs in man, as is the case with other corticosteroids.[327] This is curious since, as is discussed later, aldosterone secretion is not primarily controlled by hormones liberated by the anterior lobe of the pituitary gland. Measurement by Hernando and co-workers[311] of the amount of aldosterone in the urine of ten ambulant normal subjects, collected over 12 hours from 7 a.m. to 7 p.m., and from 7 p.m. to 7 a.m., showed that aldosterone is secreted predominantly during the day; the ratio of day excretion to night excretion found by these authors was 2.1. In only one of the ten normal subjects in this study was the day/night ratio less than unity; in one subject the ratio was as high as 8·1. The preponderance of excretion by day is not invariable. Venning, Dyrenfurth and Giroud[502] have reported observations on nine subjects, six of whom consistently excreted more by day than by night; the other three were inconsistent, excretion by night being the greater on some occasions. Muller, Manning and Riondel[334] likewise found greater excretion of aldosterone by day than by night in fifty-three out of sixty-one occasions in normal subjects. Feeding sodium chloride during the night did not alter the rhythm. Bartter and co-workers[20] noted a diurnal variation in four out of seven urines studied. Similar observations are reported by Guideri and colleagues [166a]

The existence of a diurnal variation has been denied by some investigators.[140, 502] The reason for the discrepancy between the results of different workers appears to lie in the conditions under which the observations were made. Activity has an important influence on diurnal variation. The rhythm disappears in subjects who are recumbent day and night;[337, 333] the rhythm of excretion of 17-hydroxycorticoids, however, continues unchanged. The existence of a diurnal variation seems to depend upon activity and on posture, of the two, posture appears to be the more important.[333]

The preponderance of secretion during the day is of especial interest since a similar rhythm holds for the renal excretion of sodium, i.e. is increased during the day and reduced during the night, even if the subject is maintained on a constant water and sodium intake throughout the 24 hours.[451, 444] The nocturnal retention of sodium, if this proves to be a tubular phenomenon, cannot be a result of increased aldosterone secretion (providing, of course, that this is truly reflected in the rate of renal excretion). This is further emphasized by the persistence of a diurnal rhythm of sodium excretion in cases of Addison's disease maintained only on sodium chloride The decreased excretion of aldosterone during recumbency is associated with an increased excretion of sodium, compared

with the control days when the experimental subject was ambulant by day.[344] The decreased excretion of aldosterone in the recumbent position may account for the increased sodium excretion observed when patients are confined to bed.

A diurnal variation was absent in an active, ambulant patient suffering from hypopituitarism,[337, 338] but the rhythm was restored by the administration of prednisone in doses of 25 to 30 mg. a day. The rhythm did not reappear when prednisone was administered if the patient was confined to bed throughout the 24 hours.

The diurnal rhythm of aldosterone excretion appears to be reduced during pregnancy.

Correlation between Aldosterone Excretion and Sodium Excretion in the Urine

An inverse correlation between the quantity of aldosterone in the urine and the output of sodium was noted by Luetscher and Curtis[333, 334] in a series of normal subjects on varying intakes of sodium. A direct correlation was also noted between the potassium/sodium ratio in the urine and the amount of aldosterone excreted. Similar observations have been made by Venning, Dyrenfurth and Beck,[404] Wolff, Koczorek, Buchborn and Köhler,[433] Buchborn, Koczorek and Wolff[41] and Johnson, Lieberman and Mulrow.[227] In a series of 201 individual determinations of aldosterone made by Thorn and co-workers[315, 447] on the urine of normal subjects on varying intakes of sodium, an inverse correlation between aldosterone excretion and sodium excretion was likewise noted, but only at levels of aldosterone excretion of 10 µg./24 hr. or above. Below this level the difference between means was not statistically significant.[447]

Such a correlation is, of course, to be anticipated since the relationship between administered aldosterone and the amount of sodium excreted in the urine in adrenalectomized animals is used as a method of assay. This correlation does not hold, however, under certain conditions, namely: at levels of aldosterone excretion below 10 µg /24 hr.;[447] in the case of individuals on a rapidly changing intake of sodium, as in persons at the start or finish of a period on a low sodium diet,[41] during the administration of cation exchange resins[218] or diuretics;[217] during pregnancy[241a] and in salt-losing nephritis[215, 278, 279, 447] and in patients with Conn's syndrome.

Aldosterone Excretion in Various Endocrine Disorders

The amount of aldosterone excreted in the urine of patients with various endocrine disorders has been the subject of many reports.

Following the original observation of Luetscher and Axelrad,[380] it is generally agreed that normal, or near normal, quantities of aldosterone are found in the urine of cases of *hypopituitarism*.[218, 378, 387, 411, 499] In one study[411] the mean level of excretion found in these patients was, however, significantly below the mean found in normal subjects; the mean and standard deviation in normal subjects by the method used was 5.0±3.0µg ; in nine cases of hypopituitarism the mean and S.D. was 2.9±0.4 µg./24 hr. In the single case in this report[411] in which no aldosterone could be detected in the urine, the administration of corticotrophin caused aldosterone to appear in the urine, only to disappear again when corticotrophin was withdrawn. Venning, Dyrenfurth and Beck[499] noted that a patient with hypopituitarism may have undetectable amounts of aldosterone in the urine on some occasions and detectable amounts in others. The effects of acute hypophysectomy in man on aldosterone excretion will be discussed later (p. 66).

As would be expected, patients with *Addison's disease* excrete no measurable aldosterone.[64, 215, 378, 387] In one report[218] an aldosterone excretion of 2 µg /24 hr. was found in a woman with Addison's disease during the [illegible] blank of [illegible] ulieu and [illegible]

Elevated amounts of aldosterone have been found in the urine in *Cushing's syndrome due to adrenocortical hyperplasia* by Mach,[397] and Genest.[168, 168b] Venning[498] reports the presence of increased amounts of sodium-retaining hormone in the urine of cases of Cushing's syndrome. Luetscher[378] states that aldosterone excretion in Cushing's syndrome may be either normal or high, this was also the finding of Hernando and co-workers,[215] who found consistently elevated values in only one case of the seven studied. The aldosterone excretion in four cases studied by Baulieu was within the normal range.[33a]

On the other hand, very high levels of excretion of aldosterone have been found in cases of *Cushing's syndrome due to adrenocortical carcinoma*.[46, 156, 215, 215a] An excretion of 295 µg /24 hr. was found in the case of a 7½-year-old girl who had previously undergone unilateral adrenalectomy for adrenocortical carcinoma and who also had been submitted to hypophysectomy.[218] Conn's syndrome associated with adrenocortical carcinoma has been reported;[145] the excretion of aldosterone in this case was 174 µg./24 hr Values within the normal range have also been reported in adrenocortical carcinoma.[16, 449]

An aldosterone excretion within the upper range of normal has been found in an adult with the *adrenogenital syndrome*;[215] normal values

were found in an adolescent with this disease.[218] Elevated values of urinary aldosterone have been reported in the salt-losing form of this syndrome by some,[222, 218] but complete absence by others.[41, 214, 219] The decreased secretion of corticosteroids and increased secretion of androgens in this syndrome has been ascribed to an aberration of normal synthesis of corticosteroids as the result of a defect of hydroxylation at carbon atoms 11, 17 and 21.[214] If this is so, one would expect the synthesis of aldosterone to be similarly impaired and the level of excretion to be low.

Cases have been described of adrenocortical hyperplasia in association with bronchogenic carcinoma,[243, 207, 448, 460, 222] some with and some without the clinical features of Cushing's syndrome. Such cases are characterized by the presence of hypokalaemia and alkalosis and the excretion of excessive quantities of 17-hydroxycorticoids. Aldosterone excretion in two of these patients is reported to be low (less than 1 µg /24 hr.).[207]

Normal amounts of aldosterone have been found in the urine of patients with *hyperthyroidism*,[215] *diabetes mellitus*,[214] *diabetes insipidus*,[215] *acromegaly*[21] and *phaeochromocytoma*[215] when these patients were on a normal intake of sodium and normally hydrated. Normal values have been noted in cases of *hypothyroidism*,[215] but elevated values have been reported in severe myxoedema.[224] In patients with diabetes insipidus, the withdrawal of vasopressin (pitressin) with subsequent polyuria and dehydration results in an elevation of aldosterone secretion.[215] Cases of diabetes mellitus uncontrolled by insulin with polyuria and dehydration likewise showed elevated levels of aldosterone excretion[214] which were restored to normal by the administration of insulin and rehydration. The elevated levels of excretion in both these diseases are presumably secondary to a contraction of body-water consequent upon the polyuria.

Aldosterone Excretion in Essential Hypertension

Although there has been a long-standing suppositon that the hormones of the adrenal cortex through their influence on sodium metabolism are linked with the aetiology of essential hypertension,[275] very little in the way of positive evidence has been produced to support this belief.

Genest and co-workers,[179] using a method involving immediate extraction of the urine with chloroform and subsequent hydrolysis of the remaining urine with β-glucuronidase followed by chromatography in two systems and bioassay, expressed the view that essential hypertension may be due to a state of mild chronic hyperaldosteronism. This conclu-

sion was based on the finding of elevated urinary excretion of aldosterone in six patients with malignant hypertension and seven patients with severe benign hypertension who were not oedematous and were not on sodium-restricted diets. In the method used the amount of aldosterone excreted by normal subjects is low, less than 1.4 µg /24 hr. In the hypertensive patients studied it was five to six times this amount.

In a subsequent paper Genest, Koiw, Nowaczynski and Leboeuf,[164a, 164b] using an improved physicochemical method with acid hydrolysis,[257] again found a significantly increased excretion of aldosterone in non-oedematous patients with essential benign hypertension on an unrestricted sodium intake, and in patients with malignant and renal hypertension on ward diets without added salt. The mean amount of aldosterone excreted in the urine by eleven normal subjects was 3.6 µg./24 hr., in patients with essential hypertension 7.8 µg., in renal hypertension 6.9 µg., in malignant hypertension 8.4 µg. Although the mean of the hypertensive group was double that of the normotensive group, the *aldosterone excretion of 45% of the hypertensive patients* fell within the normal range. The authors noted wide variation in the day-to-day level of aldosterone excretion when on a fairly constant sodium intake.

Leutscher[278] reported elevated levels in long-standing or malignant hypertension, but only in those cases with 'some degree' of cardiac failure. Similar results were reported by Elmadjian.[144] Venning, Carballeira and Dyrenfurth,[411] however, found increased amounts of sodium-retaining hormone in the urine of cases of malignant hypertension. A moderately increased excretion (142 to 182% of normal) of sodium-retaining hormone was found by Tronchetti, Mucio and Romanelli[410b] in patients suffering from hypertensive vascular disease.

Six non-oedematous patients with essential hypertension were studied by Hernando and co-workers[216] Of these, only two were receiving a normal sodium intake; both excreted amounts of aldosterone within the normal range. The other four were receiving a sodium intake of only 9 mEq./day; in two, the excretion of aldosterone was normal, in the other two it was elevated. The author[400] has failed to find elevated levels of aldosterone excretion in four untreated cases of essential hypertension.

From the evidence available to date, it would appear that some patients with essential hypertension excrete quantities of aldosterone which are above the normal range It is by no means certain that this is aetiologically related to the elevation of blood pressure.

Observations on a number of patients with hypertension associated with hypokalaemia suggest that, in some cases, the adrenal abnormalities may be the consequence, and not the cause, of the hypertension.[151a, 400].

[illegible] Reference to this subject is made in greater detail in the discussion of Conn's syndrome.

Aldosterone may be implicated in the 'adrenal-regeneration hypertension' described by Skelton.[illegible] Skelton noted the development of severe hypertension in young female rats which had been unilaterally adrenalectomized; the contralateral adrenal cortex had also been enucleated from its capsule leaving remnants of the *zona glomerulosa in situ*. The rats were subsequently maintained on 1% saline. Regeneration of the adrenal from this remnant is essential for the development of hypertension; prevention of adrenal regeneration by hypophysectomy or by preserving an intact contralateral gland results in failure to develop hypertension. The administration of amphenone to rats made hypertensive by this procedure resulted in a reversion of the blood pressure to normal.[illegible]

The long-term administration of a steroid spirolactone (SC-5233) following adrenal enucleation prevented the development of hypertension but did not stop regeneration of the adrenal.[illegible]

Grollman[illegible] reports that the development of adrenal-regeneration hypertension can be prevented if the initial period of hypotension due to adrenal insufficiency is avoided by the administration of cortisone and deoxycorticosterone. Masson, Koritz and Peron[illegible] think that regeneration hypertension is the result of sensitization of the vascular tree to pressor agents which takes place during the period of adrenal hypotension, similar to that occurring in Addison's disease. They found no excessive production of aldosterone from the incubated adrenal glands removed during the hypertensive phase. Pellegrino and Brogi[illegible] likewise found no excess of aldosterone in adrenal vein blood of rats with adrenal enucleation hypertension.

Excretion of Aldosterone in Cases of Congestive Cardiac Failure

The presence of increased quantities of aldosterone in the urine of patients with congestive (right-sided) heart failure is well substantiated.[22, 103, 117, 168, 170, 170, 225, 261, 262, 265, 284, 344, 361, 450, 532] What is not yet clear, however, is whether patients with this syndrome who have never received treatment with diuretics, digitalis derivatives, or dietary sodium restriction excrete quantities of aldosterone above the normal range. *Many authors have not stated the treatment received by their patients nor have they stated the sodium intake at the time the urine was collected.* The results of Wolff, Koczorek, Buchborn and Köhler[illegible] and of Wolff, Koczorek and Buchborn[illegible] suggest that there is little difference in the levels of aldosterone excretion between patients with

congestive heart failure on a normal sodium intake and those subjected to sodium restriction. The aldosterone excretion of nine out of a total of twelve 'untreated' patients (that is, patients who had received no dietary restriction or diuretics for the previous 3 weeks) was 'moderately increased' (ranging up to 35 μg./24 hr.); in the three remaining cases it was within the normal range.[131] An excretion of aldosterone within the normal range was found by Muller, Riondel, Manning and Mach[144] in five patients with congestive heart failure who had never received treatment with diuretics or digitalis derivatives and who were on a sodium intake of 100 mEq. a day; four of the five were in sodium balance, the fifth was retaining sodium. Garrod, Simpson and Tait[140] and the author[22] have noted similar findings. Three of Muller's[144] cases responded to sodium restriction by a moderate increase of aldosterone excretion, but went into negative sodium balance and lost their oedema. The fourth responded to sodium restriction by doubling his aldosterone excretion (from 15 to 33 μg./24 hr.) resulting in a positive sodium balance and increased oedema. Wolff and co-workers[138, 130, 132] record similar observations on patients with congestive failure treated by moderate sodium restriction (60–80 mEq /day). The administration of mercurial diuretics resulted in an increased excretion of aldosterone in the urine.[138] The institution of a regimen of severe sodium restriction combined with the administration of cation-exchange resins resulted in a rise of aldosterone excretion in two cases, but in a decreased excretion in another case[131] associated with rapid mobilization of oedema fluid Similar observations have been made by Duncan, Liddle and Bartter.[122] Relief of congestive heart failure is accompanied by, or results from, a return of the excretion of aldosterone to normal values.

Muller, Manning and Riondel[131] have made the interesting observation that the simultaneous administration of prednisone with a diuretic (mercurial or acetazoleamide) resulted in a decrease, rather than an increase, of aldosterone excretion, with a concommitant sodium, potassium and water diuresis. The administration of prednisone alone lowered aldosterone excretion in patients with congestive heart failure if they had previously had an elevated level of excretion.

Wolff, Koczorek and Buchborn[130] have noted that maximal renal retention of *sodium in cases of congestive heart failure was associated* with lower aldosterone levels than were found in the urine of normal subjects, submitted to sodium-restricted diets, who exhibited comparable degrees of sodium retention. This observation suggests that factors other than aldosterone are responsible for the sodium retention occurring in these patients. This view is in conformity with observations that the

suppression of aldosterone secretion by the administration of amphenone B to patients with heart failure may not result in sodium diuresis.[illegible]

No direct correlation was found between aldosterone excretion and right ventricular end-diastolic pressure or cardiac output in ten patients with congestive heart failure subjected to cardiac catheterization by Wolff and co-workers;[illegible] however, the urinary excretion of aldosterone tended to be higher in patients with a high venous pressure.

In the dog, experimental right-heart failure produced by constriction of the pulmonary artery is associated with sodium retention and an increased excretion of aldosterone in the urine[illegible] and with a higher concentration of aldosterone in adrenal vein blood[illegible] than was found in the controls. In conformity with this, hypertrophy of the zona glomerulosa of the adrenal cortex has been observed by Deane and Barger[illegible] in dogs with experimental chronic congestive heart failure. It may be concluded from these experiments that the hyperaldosteronuria of congestive heart failure results, in part at least, from an increased secretion of aldosterone by the adrenal cortex. Congestion of the liver may be an additional factor, resulting in a reduced rate of degradation.

Congestive heart failure, regardless of aetiology, is always associated with an increased vascular volume, according to Samet, Fritts, Fishman and Gournand.[illegible] Here again it is difficult in this syndrome to fit observed fact into the current theory of the control of aldosterone excretion.

The largest amounts of aldosterone excreted in patients with heart failure were found in cases of chronic tricuspid stenosis with cardiac cirrhosis, massive oedema and ascites;[illegible] one such patient excreted 126 µg /24 hr., as measured by the method of Neher and Wittstein.[illegible]

EXCRETION OF ALDOSTERONE IN THE NEPHROTIC SYNDROME

The presence of elevated amounts of a sodium-retaining factor (proved to be aldosterone[illegible]) in the urine of patients with the nephrotic syndrome has been reported by many authors.[11, 103, illegible] Singer[illegible] has also reported an elevated concentration of aldosterone in blood from the adrenal vein of rats with experimentally produced nephrosis; the administration of cortisone and prednisolone to these rats reduced aldosterone secretion to normal.[illegible] Many of these authors claim a direct correlation between the degree of oedema present and the output of aldosterone. McCall and Singer[illegible] noted the presence of sodium-retaining factor (estimated by bioassay) in the urine of nephrotics when they were accumulating oedema fluid, and its absence when these patients were undergoing a diuresis. Luetscher, Deming and Johnson[illegible] noted

that successful treatment of the syndrome with cortisone or corticotrophin, promoting a sodium diuresis and loss of oedema, is associated with a fall of aldosterone excretion towards, or to, normal. Corticotrophin *may first produce a transitory increase* of aldosterone excretion, with weight gain. The administration of prednisone may likewise be followed by a decreased excretion of aldosterone, with concomitant sodium and water diuresis.[513, 514]

The administration of human albumin to some cases of the nephrotic syndrome is likewise associated with a prompt fall of aldosterone excretion and a sodium diuresis in some cases.[406] A similar finding is reported by Luetscher[379] and by Bartter.[30] In other cases, no response was seen.[406]

Hyperaldosteronism has also been noted in a patient with oedema due to idiopathic hypoproteinaemia.[379]

Aldosterone Excretion in 'Salt-losing Nephritis' and other Renal Lesions

Elevated values have been reported in patients with so-called 'salt-losing nephritis'.[315, 376, 379] The sodium intake at the time of the urine collection is not stated in many of these papers. However, the sodium intake of two patients studied by Hernando and co-workers[316] was commensurate with the urinary excretion; both had high levels of aldosterone in the urine (60 and 42 μg./24 hr.).

A high urinary excretion of aldosterone has also been noted in patients with polycystic disease of the kidneys.[84] Elevated values have been found in four cases of renal osteodystrophy with polyuria.[406] These patients showed an inability to respond to the administration of deoxycorticosterone by reduction of sodium excretion in the urine, i.e. they were 'salt-losers'. No observations are available on aldosterone excretion in cases of acute nephritis.

Aldosterone Excretion in Hepatic Cirrhosis

Increased quantities (up to twenty times normal) of a sodium-retaining factor with the characteristic of aldosterone have been reported by many authors[42, 43, 117, 131, 161, 204, 262, 287, 471, 487, 520, 530, 531, 533] in the urine of patients with cirrhosis accompanied by ascites, but not necessarily by oedema. Patients with both acute and chronic hepatitis without ascites have been stated to excrete normal amounts of aldosterone, although there is one report[316] of elevated values (up to three times normal) in these conditions. The increased reabsorption of sodium in the colon[38] and reduced sodium/potassium ratio in saliva, sweat[137] and urine[533] in cirrhosis suggests elevated concentrations of circulating aldosterone in

these patients; this has now been confirmed experimentally by Lobotsky, Buss Hannye and Lloyd.[211]

A decreased rate of destruction in a diseased liver is a possible cause of the elevated plasma levels and increased urinary output rather than an increased rate of secretion by the adrenal, since Chart, Shipley, Helmer and LeSher,[411] Gordon[177] and Reaven[311] have demonstrated that aldosterone is inactivated by liver slices *in vitro*. Wolff, Koczorek and Buchborn[311] recovered 15% of an aldosterone load (500 μg.) in the urine of patients with cirrhosis, but only 4.6% in the urine of normal subjects; this would support the suggestion of a decreased rate of degradation in cirrhotic patients. Venous congestion of the liver produced by constriction of the inferior vena cava above the level of the hepatic vein was found by Yates[311a] to result in impairment of the renal excretion of sodium, suggesting that inactivation of aldosterone may be reduced by venous congestion of the liver. In a further paper, Yates, Urquhart and Herbst[311b] found that enzymic reduction of ring A of cortisone, hydrocortisone, deoxycorticosterone and probably of aldosterone is impaired in livers from rats with acute venous congestion of the liver. Nevertheless, in this connection it should be noted that the plasma levels and urinary excretion of 17-ketosteroids and 17-hydroxycorticoids in liver disease are not raised, but are normal or even low.

A fall of aldosterone excretion was observed by Dyrenfurth, Stacey and Venning[121] to follow abdominal paracentesis, with a gradual build-up in excretion until the next paracentesis. The reverse has been reported by Wolff, Koczorek and Buchborn,[310, 311, 312] namely a considerable increase in the level of aldosterone excretion, and diminution of sodium excretion, following paracentesis. Both types of response have subsequently been reported by Dyrenfurth, Stacey, Beck and Venning.[illegible] They distinguished three phases of aldosterone excretion following abdominal paracentesis. For the first 2 days there may be a rise, a fall or no change in *aldosterone excretion. As the ascitic fluid reaccumulated there was a rise* of aldosterone excretion in all their patients, this phase lasted up to 6 days. Following this there was a fall of aldosterone excretion until the next paracentesis In an attempt to account for these changes Wolff and co-workers[310] made a study of internal shifts of sodium and water during the period following paracentesis. Following paracentesis there was loss of sodium and water from the plasma into the peritoneal cavity as indicated by a transient decrease of plasma volume. A compensatory shift of sodium and water occurred from the intracellular to the extracellular compartment. These changes were accompanied not only by alteration of secretion of aldosterone, but also of antidiuretic hormone.[311]

The administration of prednisone to cirrhotic patients with ascites is reported by Vesin and colleagues[58, 515, 516] to result in a fall of aldosterone excretion to normal and in a diuresis of sodium and water. The mechanism whereby this is achieved has yet to be explained.

The concentration of aldosterone in ascitic fluid has been reported as 0.09 to 0.233 µg./100 ml.;[121] unfortunately comparative values are not given for blood and urine in these cases.

Aldosterone Excretion during Pregnancy

The urinary excretion of aldosterone is raised during pregnancy. Bioassay of sodium-retaining factor by Chart, Shipley and Gordon,[145] Venning, Singer and Simpson[608] and Gordon, Chart, Hagedorn and Shipley[170] showed that women with pre-eclampsia excreted quantities of aldosterone which may reach ten times the upper limit of normal. In *uncomplicated pregnancies the level of excretion of aldosterone was found* by these authors to be within the normal range or only slightly above. *Barnes and Quilligan,*[27] however, later reported that the excretion of sodium-retaining factor in *pregnancy* was raised by 50 to 150% above normal, and found no difference between patients with toxaemia and *those without Values for aldosterone excretion of up to five times normal* were found by Martin and Mills,[410] using a physicochemical method, in fifty-five normal pregnancies, in twenty patients with toxaemia no values were found which exceeded those found in a normal pregnancy. Venning and Dyrenfurth[607] and Venning, Primrose, Caligaris and Dyrenfurth,[607] using a method involving a single chromatographic separation and bioassay, followed aldosterone excretion during pregnancy and found a steadily progressive rise as term approached. Venning's[607] patients, however, were placed on 'moderate salt restriction' during the third trimester. Martin and Mills,[418] Koczorek, Wolff and Beer,[341a] Wolff, *Koczorek and Buchborn*[339] *and Rinsler and Rigby*[503] *similarly report a* progressive increase to term. Wolff and *co-workers*[341a, 339] *found levels* excreted by pregnant women which were up to six times those found in non-pregnant women. Following delivery there was a rapid fall to normal Both Martin and Mills[411] and Wolff and co-workers[341a, 339] found that toxaemia was not associated with levels of aldosterone excre-

n all *eleven preg-*

est,[338, 337] in one

case the output was 200 µg./24 hr. Hernando and co-workers found a *value of 58 µg./24 hr* in the second trimester, and another of 28 µg in

the third trimester of uncomplicated pregnancy[113] and a value of 10 µg. in a case complicated by toxaemia.

Observations on aldosterone excretion in pregnant women are also reported by Kumar, Feltham and Gornall.[111a] Elevated values were found when the patients were on a daily intake of sodium of 140 mEq.; in six patients mean values of 49 µg./24 hr. were found, compared with 8 µg./24 hr. for non-pregnant women of comparable age on a similar diet. On a sodium intake of 20 mEq./day, the excretion of aldosterone in the pregnant women rose to a mean of 101 µg /24 hr. Five patients with pre-eclampsia excreted a mean of 25 µg. of aldosterone daily when on a normal sodium intake.

An increased rate of secretion of aldosterone in some women during pregnancy has been found by Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook and Greenwood,[111b] by a method of isotope dilution using tritiated aldosterone. The secretion rate was elevated in 4 of the 6 patients studied in the 32nd to 38th week of pregnancy; in one case the rate was five times that found by the same authors in a series of six non-pregnant women.

An excretion of only 2 µg /24 hr. was found in the third trimester of pregnancy in a patient with Addison's disease by Hernando and co-workers.[115] A 'negligible' amount of aldosterone was found by Gordon and co-workers[179] in the urine of a pregnant woman suffering from Addison's disease. The complete absence of aldosterone from the urine under these circumstances was also noted by Baulieu, de Vigan, Boicaire and Jayle[12] and by Christy and Jailer.[41b] On the other hand, Laidlaw, Cohen and Gornall[111] report finding 4.4 µg. of aldosterone in the urine of a bilaterally adrenalectomized woman during the 38th week of pregnancy; none could be detected post-partum. These observations render it unlikely that aldosterone is secreted by the placenta, although claims have been made for the isolation of this steroid from placental extracts.[170, 415, 416] The steroid in these cases may have been concentrated in the placenta but originated in an extraplacental site.

It has been suggested that the increased amount of aldosterone found in the urine during pregnancy may not reflect a true elevation of secretion, but rather a reduced rate of metabolic degradation of the hormone in the liver, leading to an increased percentage of secreted aldosterone being excreted in the liver, as occurs in the case of cortisol during pregnancy. However, direct evidence that the secretion rate is raised in some instances has been provided by Jones and colleagues.[111b] In addition, these workers found an alteration in the pattern of metabolism of aldosterone during pregnancy. There was a great increase in the amount excreted in the form of a conjugate which is hydrolysed by acid hydrolysis at pH1, and a

decreased conversion to metabolites conjugated with glucuronic acid. These results confirm that there are both quantitative and qualitative [illegible] progesterone.[illegible]

ALDOSTERONE EXCRETION FOLLOWING SURGICAL OPERATIONS

An increase in the amount of a sodium-retaining factor excreted in the urine in the 24 hours following surgical operations was reported by Llaurado.[illegible] The sodium-retaining factor was estimated by bioassay of crude chloroform extracts of unhydrolysed urine. Later Llaurado, Neher and Wettstein[illegible] identified the sodium-retaining factor as aldosterone. Llaurado and Woodruff[270] studied 'aldosterone' excretion, measured as above, in the urine during the *first 3 post-operative days and* found that it was increased during this period. They also noted a significant correlation between the reduction of the sodium/potassium ratio in the urine of the test animals caused by the injection of the urine extract and the logarithm of the sodium/potassium ratio in the patient's urine from which the extract was prepared. By the time the sodium/potassium ratio of the patient's urine had returned to its pre-operative level (5th–15th day) *the aldosterone activity of the urine extracts had also returned to control* values. To account for these findings, Llaurado and Woodruff[illegible] suggested that the rate of destruction of aldosterone may be diminished after operation as the consequence of impairment of liver function.

Casey, Bickel and Zimmerman[illegible] and Zimmerman, Casey and Bloch[illegible] also found a raised excretion of aldosterone during the first 24-hour period following surgical operations. Aldosterone was measured in these studies by β-glucuronidase hydrolysis of urine, benzene-water partition of the chloroform extract, chromatography in the toluene-propyleneglycol system and bioassay. They conclude that the increased excretion of aldosterone persists for too brief a period to be responsible for the prolonged sodium retention which follows surgery. A similar conclusion was reached from observations on patients undergoing total adrenalectomy when maintained on a constant dose of cortisone throughout the immediate post-operative period.[illegible]

The aldosterone excretion of three patients undergoing gastrectomy was studied by Venning, McCorriston, Dyrenfurth and Beck.[illegible] Two of the three patients showed an increased excretion of aldosterone on the day of operation only; in the third the raised excretion persisted for 6 days. Only one of these cases showed a significant degree of sodium retention on

the day of operation, but all three showed sodium retention later in the post-operative period when the excretion of aldosterone in two of the three cases had fallen to normal. All showed a prolonged increase of excretion of 17-hydroxycorticoids.

Wolff and Koczorek[451] present a graph showing aldosterone excretion following operation; the number of cases is not stated. The maximum excretion was achieved on the third and fourth post-operative days and reached levels five times those of control values.

It must be added that no significant increase of aldosterone excretion was observed by Ross and co-workers in a single case.[411] It is probable that the degree of blood loss and efficiency of its replacement plays a large part in determining the extent of the aldosterone response to surgery.

B. The Concentration of Aldosterone in Blood

The presence of a substance with sodium-retaining properties was noted in 1950 by Spencer[431] in adrenal vein blood of the dog. A sodium-retaining factor, subsequently identified as aldosterone, was extracted and isolated chromatographically by Simpson, Tait and Bush[441] from adrenal vein blood of the dog and monkey. Wettstein and Anner[449] have reported finding a concentration of aldosterone of 0.35 μg./100 ml. in beef blood. In fresh beef adrenals they had found 40–95 μg. of aldosterone per kilogram; they commented that the quantity of circulating aldosterone in this animal was about seventy times that of the total content of its adrenal glands.

Farrell and collaborators[137, 140, 141, 143, 144] have identified aldosterone in adrenal vein blood from intact and hypophysectomized dogs. The concentration was such as to suggest a rate of secretion of 0.22 to 0.51 μg /kg. body wt./hr. in four control dogs and of 0.06 to 0.19 μg./kg /hr. in four hypophysectomized dogs. No aldosterone could be detected in the peripheral blood of the dog by Davis, Pachet, Ball and Goodkind,[88] but concentrations of up to 2.8 μg./100 ml. were found in adrenal vein blood of intact dogs Adrenal vein blood of dogs with experimentally induced congestive heart failure contained up to 7 0 μg./100 ml. and that of dogs with inferior venal caval obstruction up to 12.4 μg /100 ml.[107] The blood loss involved in these experiments will stimulate aldosterone excretion.

Sodium-retaining activity (presumed due to aldosterone) has likewise been identified in adrenal vein blood of rats by Singer and Stack-Dunne [447, 440] and its rate of secretion estimated at 45 μg. deoxycorticosterone equivalents per adrenal per kilogram body weight per hour An increased

rate of secretion into adrenal vein blood was found in nephrotic rats [444]

In man, Simpson and Tait[440] found levels of 0.02 to 0.2 µg./100 ml. in pooled samples of peripheral plasma. Later, Ayres and collaborators [441] report concentrations of 0.03 to 0.14 µg./100 ml. of plasma in one subject on a normal diet, and less than 0.10 µg./ml. in another. In a subject on a sodium-restricted diet a mean level of 0.4 µg./100 ml. was found. However, calculations based on estimations of the secretion rate, biological half-life and miscible pool of aldosterone in man give a mean plasma concentration over the 24 hours, neglecting diurnal variation, of 0.03 µg /100 ml.,[172] which is in fair agreement with the above results. Because of the low concentrations involved, these measurements cannot be very accurate, but they give some indication of the very low levels of circulating aldosterone existing in the peripheral circulation. No figures are available at present for the concentration of aldosterone in adrenal vein blood in man.

Excretion of Aldosterone in Bile and Faeces

No information is at present available as to the excretion of aldosterone in bile or faeces.

BIOSYNTHESIS AND METABOLISM OF ALDOSTERONE

THE isolation of aldosterone from adrenocortical extract,[146, 149, 144] from adrenal vein blood,[141] from fresh beef[141] and hog adrenals,[140, 149, 151] and its higher concentration in adrenal vein blood than in peripheral blood[14] all point to the adrenal gland as the site of origin of aldosterone. This is supported by its absence from the urine of patients with Addison's disease and following bilateral adrenalectomy. Direct confirmation of an adrenal origin has been provided by *in vitro* studies [10, 171, 170]

FUNCTIONAL ZONES OF THE ADRENAL CORTEX

The chance observation of Ayres, Gould, Simpson and Tait[10] that decapsulation of beef adrenal glands, which strips the zona glomerulosa from the gland, removed their ability to secrete aldosterone on incubation has led to the recognition, at least in certain species, of a clear-cut functional 'zonation' of the adrenal cortex with respect to the site of production of aldosterone. A similar finding in rat adrenal glands has been reported by Giroud, Stachenko and Venning.[170]

The initial observations were extended by Giroud, Stachenko and Piletta,[172] using fresh beef adrenal glands from which the zona glomerulosa had been pared by means of a razor blade. Slices of the zona glomerulosa and of the remaining two zones were incubated in Krebs-Ringer bicarbonate-glucose medium. Aldosterone was produced only by the zona glomerulosa, at a rate of 26 µg./100 g. tissue/hr. Cortisol was produced by the zona fasciculata and zona reticularis; corticosterone by all three zones in about equal amounts, weight for weight. These observations may indicate that 17-hydroxylase is absent in the zona glomerulosa, and that 11- and 21-hydroxylase are present in all three layers.[172]

These data confirm previous indirect evidence obtained from histological studies which had led to the belief that the zona glomerulosa of animals was concerned with the secretion of a mineralocorticoid, based on the observation that restriction of sodium intake in the rat results in widening of the zona glomerulosa,[109, 211, 164] on the cytological changes occurring in the zona glomerulosa of the rat when treated with deoxycorticosterone[184] and on the persistence of the zona glomerulosa and of aldosterone secretion following hypophysectomy.[97]

In man, the evidence in favour of the zona glomerulosa as the layer responsible for the secretion of aldosterone is not so clear cut. Neher[241] reports a case of Cushing's syndrome due to hyperplasia who also had carcinomatosis (the site of the primary lesion is not stated). The zona fasciculata had been replaced by tumour, but the zona glomerulosa remained. Analysis of this gland showed a high content of aldosterone [illegible]

terone to the zona glomerulosa is given on p. 60.

The morphology of adenomas removed from cases of Conn's syndrome does not show the consistent preponderance of any one zone. In some cases the cells resemble those of the zona glomerulosa, in others, those of the zona fasciculata. Atrophy of the remaining gland in some cases is confined to the zona fasciculata, in others to the zona glomerulosa; in other cases no atrophy was present. This variation in the morphology of Conn's syndrome is not entirely unexpected, since the cause of this syndrome is almost certainly not solely the result of excessive aldosterone secretion.

PRECURSORS OF ALDOSTERONE

Experimental studies of the pathways of aldosterone biosynthesis are as yet too fragmentary for it to be possible to formulate a definite scheme of precursors. A tentative scheme put forward by Pincus[245] suggests a pathway by way of cholesterol, pregnenolone, progesterone and deoxycorticosterone. Fig 2 shows possible pathways in the biosynthesis of aldosterone.

The secretion of aldosterone by homogenates of beef adrenal glands was found by Wettstein, Kahnt and Neher[240] to be increased by the addition of deoxycorticosterone and decreased by the addition of progesterone and corticosterone. Later,[241] they showed that 21-^{14}C-deoxycorticosterone could be converted to 21-^{14}C-aldosterone by beef adrenal homogenates, in a yield of 48% of theoretical maximum. These observations suggest that progesterone is not an obligatory intermediary whereas deoxycorticosterone may be.

Rosemberg, Rosenfeld, Ungar and Dorfman,[246] using perfused calf adrenals, found that progesterone significantly increased the yield of aldosterone; corticosterone and deoxycorticosterone did not. The conversion of ^{14}C-progesterone to ^{14}C-aldosterone by perfused calf adrenals was later confirmed.[244] These findings with perfused glands are thus at

variance with those of Wettstein and collaborators[212, 219] using homogenates. However, beef adrenal capsule strippings (i.e. zona glomerulosa) were found by Travis and Farrell[400] to be capable of converting ^{14}C-progesterone into ^{14}C-aldosterone, whereas ^{14}C-corticosterone was not converted into aldosterone.

Giroud, Stachenko and Piletta[213] demonstrated by their slice technique that aldosterone production in slices of the zona glomerulosa was increased by deoxycorticosterone, 11-hydroxyprogesterone and corticosterone equally. These substances do not result in the production of aldosterone when added to slices of combined zonae fasciculata and reticularis.

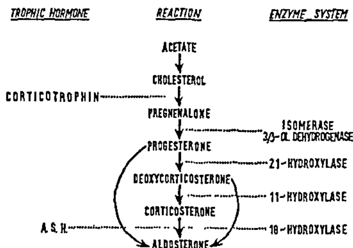


Fig 2 Suggested pathways of aldosterone biosynthesis
(A S H. = 'aldosterone stimulating hormone')

Wettstein, Kahnt and Neher[220] found that the quantity of aldosterone secreted by homogenates of beef adrenal glands, incubated aerobically, was greatly increased by the addition to the medium of nicotinamide, fumaric acid, glucose and adenosine triphosphate (ATP). The secretion of aldosterone by homogenates of hog adrenal glands, which produced ten times as much aldosterone as beef adrenal glands, was little affected by the addition of these supplements.

Acetic acid, which accelerated the biosynthesis of corticosteroids,[219] greatly reduced the amount of aldosterone synthesized by beef adrenal homogenates.[220]

The Rate of Secretion of Aldosterone by the Human Adrenal Cortex

The rate of secretion of aldosterone by normal subjects has been determined by Ayres and collaborators,[16] using a method of isotope dilution. *Following the injection of 16-^{3}H-aldosterone intravenously, the specific* activity of the aldosterone excreted in the urine during the next 24 hours was determined; from this the dilution of injected aldosterone can be calculated. Assuming that there is no store of aldosterone in the body, this represents the amount of aldosterone secreted during the collection period, i.e. in 24 hours. When on a normal sodium intake, the total amount of aldosterone secreted daily by one normal subject was estimated to be 170 μg., that of another 190 μg./24 hr. On a low sodium intake the rate of secretion of the second subject rose to 780 μg./24 hr. The amounts of aldosterone appearing in the urine of this patient on these two levels of sodium intake were 7.2 and 42 μg./24 hr. respectively.

A slightly different isotope dilution method, based on measurement of tetrahydroaldosterone excreted in the urine, was employed by Ulick and colleagues[210a] to estimate the secretion rate of aldosterone. On a normal sodium intake the secretion rate of a normal subject was 230-350 μg /24 hr, rising to 930 μg /24 hr. on a restricted sodium intake. These figures are in satisfactory agreement with those of Ayres.[16]

Aldosterone secretion rates have also been measured by Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook and Greenwood[210b] The secretion rate varied from 82-190 μg./24 hr. (mean = 114 μg) in 4 normal men, and from 72-315 μg /24 hr. (mean = 192 μg) in 6 normal females.

Biological Half-life of Aldosterone in Man

A provisional figure for the biological half-life of aldosterone in man, a measure of its rate of degradation, has been calculated from a very *restricted number of observations of the decay of injected 16-^{3}H-aldos*terone in the blood, and is given by Ayres and co-workers[16,17] as 0.4 hours (= 24 minutes). Comparable figures for cortisol and corticosterone are 1.4 hours and 1.0 hours respectively A biological half-life for aldosterone of 50 minutes is given by Kliman and Peterson,[137] using tritiated aldosterone.

Inhibition of Aldosterone Secretion by Amphenone and Other Substances

Amphenone B (3,3-bis(p-aminophenyl) butanone-2) synthesized by Allen and Corwin[4] as a substance likely to possess progestational activity, has been shown to interfere with the biosynthesis of adrenocortical

hormones[illegible] possibly by blocking hydroxylation at the 11, 17 and 21 positions.[illegible]

[illegible] apparent, and a lag of 24 hours before the reappearance of aldosterone in the urine when the administration of amphenone is stopped. Under certain circumstances (cases of Cushing's disease) cessation of amphenone administration was followed by a striking temporary increase in the amount of aldosterone excreted,[illegible] suggesting that a store of precursors of aldosterone had accumulated in the adrenal cortex during the period of amphenone administration and that large quantities of aldosterone were formed from these precursors and quickly released when the block to synthesis was removed. ✓

Amphenone has been observed to cause a striking decrease in the excretion of aldosterone in two patients with adrenal carcinoma.[illegible] In one of these patients an escape from the inhibitory effect of amphenone was seen during a prolonged period of amphenone administration. The administration of corticotrophin can likewise partly overcome the inhibitory effect of amphenone.[illegible]

Coincident with the suppression of aldosterone secretion during the administration of amphenone there is, in most cases, a sodium diuresis of varying magnitude. This has been seen in normal subjects during dietary sodium restriction[illegible] and in some cases of secondary hyperaldosteronism due to congestive heart failure,[illegible] and cirrhosis.[illegible]

The administration of amphenone was used as a diagnostic tool of great value in the case of a patient with gross oedema associated with an aldosterone-secreting adrenocortical adenoma[illegible] and has been so used in a case of Conn's syndrome.[illegible]

[illegible] a causative factor. Unfortunately amphenone B is too toxic to administer for periods of more than 2 or 3 days. ✓

The administration of Δ^4-cholesterone, an inhibitor of cholesterol synthesis, is reported[illegible] to reduce the production of steroids by rat adrenal glands. The amount of corticosterone secreted into adrenal vein blood was reduced to 20% of that secreted by the controls; the amount of aldosterone in two pooled samples of adrenal vein blood from control animals was 5.6 and 6.1 µg, in blood from the treated group 3.5 and 4.2

µg. per 100 ml. The secretion of aldosterone was thus reduced to a much more limited extent than was that of corticosterone.

Degradation Products of Aldosterone

Little is known of the forms in which aldosterone is excreted in the urine. Following the injection of tritiated aldosterone, Ayres and colleagues[17a] recovered 33% of the injected radioactivity from the urine [illegible]

the steroid. Evidence for the presence of *tetrahydroaldosterone* in urine has been adduced by Ulick and Lieberman.[431e] Only 0.2% of the injected dose was excreted as free aldosterone. Ten per cent of the administered radioactivity was extracted by chloroform after hydrolysis at pH 1 for 24 hours; of this, half was in the form of free aldosterone.

Information on the forms in which aldosterone is excreted is also provided by Jones and colleagues.[230e] They emphasize that the steroid is excreted in part as a Δ^4,3-keto conjugate which is split by acid hydrolysis to yield free aldosterone. This fraction amounts to some 10-15% of the amount of aldosterone secreted by the adrenal gland, when allowance has been made for recovery rate of the method and efficiency of hydrolysis. Part of the secreted aldosterone is *excreted* as a *metabolite*, probably tetrahydroaldosterone, conjugated with glucuronic acid This fraction amounted to about 39% of the injected radioactivity in this study. Very little free aldosterone was extracted from urine at neutral pH. This *fraction amounted to only 0.24% of the injected radioactivity.*

The change of pattern of excretory forms during pregnancy has been referred to in Chapter III. There is no reason to conclude that this is the only circumstance in which the metabolic degradation of aldosterone is altered.

Binding of Aldosterone by Plasma Proteins

Little information is available concerning the binding of aldosterone by plasma proteins. Slaunwhite and Sandberg[454b] have published evidence for the presence of a protein fraction ('transcortin') in plasma which is highly active in binding steroids, but no mention is made of aldosterone *in their paper* Daughaday[47b] *found that aldosterone had only weak* activity in displacing cortisol and corticosterone from binding sites on plasma proteins.

Renal Clearance of Aldosterone

No information is currently available concerning the threshold and handling of aldosterone by the renal tubules. .

CHAPTER V

BIOLOGICAL PROPERTIES OF ALDOSTERONE

Effect on Renal Excretion of Sodium and Potassium

The discovery of aldosterone was the direct consequence of observations upon the renal excretion of sodium and potassium by the adrenalectomized animal following the administration of aldosterone-containing extracts. Estimates of its potency in causing reduction of the excretion of sodium and promoting the excretion of potassium vary with the type of assay used. It is customary to use the readily available compound, 11-deoxycorticosterone, either the acetate or the free alcohol, as reference standard for purposes of comparison, since its actions on the kidney closely resemble

Table III

ACTIVITY OF 'SODIUM-RETAINING FACTOR' AS DETERMINED BIOLOGICALLY BY ALTERATION OF THE RATIO OF SODIUM TO POTASSIUM EXCRETED IN THE URINE OF ADRENALECTOMIZED RATS, COMPARED WITH DEOXYCORTICOSTERONE AS REFERENCE STANDARD

Reference	*Method of assay (reference)*	*DOC* equivalents µg/24 hr.*	*DCA† equivalents µg/24 hr.*
Speirs *et al* [449]	437	121 ± 8	
Mattox *et al* [330]	modification of [437]	c 100	
Farrell *et al* [144]	144	46	
Simpson *et al.* [443]	437	100	
Llaurado *et al.* [367]	367		100
Ross [406]	406	25–50	

* Expressed in terms of activity of deoxycorticosterone as reference standard.

† Expressed in terms of activity of deoxycorticosterone acetate as reference standard.

those of aldosterone. Using the ratio of sodium to potassium present in the urine as an index of activity, estimates of potency of aldosterone in terms of deoxycorticosterone or deoxycorticosterone acetate are shown in Table III.

The effect of aldosterone (at least at higher dosage levels) in causing sodium retention is more pronounced than its effect in promoting potassium excretion. Estimates of its activity compared with deoxycorticosterone, when one or other or both of these criteria are used have been made by various workers, with the results shown in Table IV.

It is generally agreed that the effects of aldosterone on the handling of electrolytes by the renal tubule are, in general, qualitatively similar to those of deoxycorticosterone, but that it exerts these effects in much smaller doses than does deoxycorticosterone. There are, however, certain differences between the actions of the two steroids as detailed below.

In the adrenalectomized rat, aldosterone (in doses of 1 and 10 µg per rat) caused pronounced sodium retention under conditions of a hypotonic saline load,[105] but its effectiveness diminished as the tonicity of the salt load increased. It thus resembles to some extent the action of deoxycorticosterone in this respect, but with the difference that deoxycorticosterone is entirely without effect under conditions of a hypertonic salt load.[105] The extent of the potassium diuresis due to aldosterone is proportional to

TABLE IV

ACTIVITY OF 'SODIUM-RETAINING FACTOR' AS DETERMINED BY ITS EFFECT ON THE EXCRETION OF SODIUM OR POTASSIUM IN THE URINE OF ADRENALECTOMIZED RATS OR IN PATIENTS WITH ADDISON'S DISEASE

Reference	*Test animal*	*Method of assay (reference)*	*Effect on sodium excretion**	*Effect on potassium excretion**
Simpson *et al.*[442]	Rat	437	50× DOC	
Axelrad *et al.*[10]	Rat	228	50× DOC	
Desaulles *et al*[109]	Rat	232	25× DOC	5× DOC
Johnson *et al.*[228]	Rat	226	20–30× DOC	
Mach *et al*[301]	Man		70× DOC	
Prunty *et al*[374]	Man		10× DOC	
Farrell *et al*[141]	Rat	144	25× DOC	
Muller *et al*[395]	Man		20× DOC	

* Determined by comparison with activity of deoxycorticosterone (DOC) as reference standard.

the degree of sodium retention attained under these conditions.[105] The intravenous administration of isotonic saline to intact rats results in a diuresis of sodium due to decreased sodium reabsorption by the renal tubules. This increased excretion of sodium could not be prevented, although it was reduced, by the administration of 10 µg. of aldosterone, given intravenously 30 minutes before the infusion of saline commenced.[65]

The ability of dogs to excrete a hypertonic saline (2.5% NaCl) load was found to be impaired by adrenalectomy.[444] The infusion of aldosterone, in doses of 23, 30 and 40 µg., had no effect on the amount of sodium excreted by the salt-loaded adrenalectomized dogs. There was, however, an increase in the amount of potassium excreted.

Overdosage with aldosterone (1000 µg. of DL-aldosterone acetate per

day) in adrenalectomized dogs did not result in as much sodium retention as occurred with 700 µg. of deoxycorticosterone acetate per day.[193] No alterations in sodium and potassium concentrations in the plasma were noted with this dosage of DL-aldosterone acetate and the haematocrit remained constant.[193]

Aldosterone, in amounts equivalent to 1.94 µg./min. of the free alcohol, resulted in a pronounced decrease of sodium excretion and in a much less pronounced increase of potassium excretion when infused intravenously in three patients with Addison's disease.[416] During the last 6 hours of the 8 hour infusion, the amounts of sodium excreted on the control and experimental days were respectively: 69 9 and 18.3 mEq.; 53.8 and 16.2 mEq.; 65.1 and 19 8 mEq. The corresponding figures for potassium excretion were: 27.4 and 40.5 mEq ; 37.2 and 37.3 mEq.; 20.2 and 28.9 mEq. During the first two hours of the infusion, little change in electro- [illegible]

control and experimental days were: 48.6 and 18.9 mEq. for sodium excretion and 9.5 and 24.2 mEq. for potassium excretion in one subject; 76 4 and 35.7 mEq. for sodium, 5.9 and 12.6 mEq for potassium excretion in the other subject.

The prolonged administration of aldosterone to normal subjects, at a dosage level of 3 to 6 mg. of DL-aldosterone monoacetate daily for 13 days in one subject and 26 days in another, resulted in sodium retention, increased potassium loss in both urine and stools, and weight gain.[74] After approximately two weeks, the weight reached a maximum and then declined towards control values. Sodium excretion rose again, after 4 days in one subject and after 16 days in the other, to reach control levels. There was little decrease in the excretion of potassium when sodium excretion was reverting towards control values. Cessation of aldosterone administration was followed by a prompt sodium diuresis, potassium retention and return of weight to control values. In one subject, the mean daily figures for sodium excretion in urine and faeces were as follows· 7 day control period, 107 mEq ; 13 day experimental period, 85 mEq , 4 day period after stopping aldosterone administration, 232 mEq. The corresponding figures for the second subject were 59, 106 and 133 mEq. For potassium, the figures for mean daily excretion in urine and faeces were 98 5, 110 and 60.2 mEq for the first subject, and 73.5, 92 7 and 36 8 mEq. for the second subject.

Coincident with the changes in the rate of excretion of sodium and

potassium during aldosterone administration, there is a decreased excretion of chloride ion and an increased excretion of hydrion (titratable acid) and of ammonium ion.[258]

Time Relationships of the Effect of Aldosterone on Electrolyte Excretion

The time relationships of the effect of aldosterone on renal tubular control of sodium and potassium excretion are of some interest. Graphs published by investigators who have administered aldosterone orally, intramuscularly or intravenously to man, and collected the urine samples over short periods of time, reveal that its effect on the excretion of electrolytes is not immediate. A delay of 1 to 2 hours is found before there is a noteworthy effect on sodium excretion.[28, 82, 338, 410, 466] In the Liddle[361] method of bioassay involving intravenous injection to the adrenalectomized dog, a delay of 1 hour occurs before there is any appreciable effect on electrolyte excretion. An immediate effect on sodium excretion, but a 2-hour delay before there is an effect on potassium excretion, is reported by Davis[82] following intravenous injection of 16 μg. of aldosterone to an adrenalectomized dog. Barger and co-workers[35, 36] on the other hand, found that small doses of aldosterone (10 μg. over 2 hours) infused directly into the renal artery of an unanaesthetized dog with intact adrenal glands had no effect on sodium excretion, but caused a potassium diuresis. If the dog was now adrenalectomized, and time allowed for recovery from this procedure, the infusion into the renal artery of only 0.45 μg. of aldosterone over 100 minutes caused sodium retention, with a potassium diuresis of the same dimensions as before. In all Barger's animals there was a delay of approximately 60 minutes before any effect on electrolyte excretion was observed. The effect of the injection of aldosterone into a peripheral vein was similarly delayed Maximum sodium retention and potassium excretion occurred 2 to 3 hours after commencing the infusion.

DL-aldosterone monoacetate has been administered intravenously to three patients with Addison's disease by Ross, Reddy, Rivera and Thorn[416] at a rate of 4.2 μg./min. over an 8-hour period. This is equivalent to a rate of 1.94 μg./min. of D-aldosterone as the free alcohol; the rate of secretion of D-aldosterone by the human adrenal cortex under normal conditions has been estimated to be 0.14 μg./min[416] The amount administered was considered to represent a maximal stimulus; despite this, in no case was an effect demonstrable within the first 2-hour period of the infusion Maximum depression of the urinary sodium/potassium ratio was observed 8 to 12 hours after the commencement of the infusion.

A delay of six hours before sodium retention commenced was noted by

Dingman and colleagues[114] during the intravenous administration of 1 mg. of aldosterone over a period of 24 hours to a normal subject.

It may be significant that a similar delay has also been noted before the effect of intravenously administered deoxycorticosterone acetate[118] and of 9α-fluorohydrocortisone[111] on sodium excretion becomes apparent.

It is difficult to account for the time lag when aldosterone is given intravenously, and even more so when it is given directly into the renal artery. A possible cause of delay could be difficulty of penetration of the steroid to its site of action within the tubular epithelial cell, but aldosterone is a lipoid soluble substance and should readily penetrate a lipoprotein cell membrane. A second possibility is that the delay is due to the slow adaptation of the enzyme system responsible for the electrolyte transport to the presence of the steroid, so that some time elapses before the enzyme is present in amounts adequate to produce a measurable alteration in urinary electrolyte concentration. It is possible that aldosterone, given as the free alcohol or the acetate, may have to be changed into a different chemical form before it is active, and finally aldosterone itself may be the sodium carrier and that a sufficient concentration may have to built up at a specialized site before it can be effective as a transport system.

The absence of an effect on sodium excretion in Barger's[14] normal dogs is even more difficult to explain. It may be that the normal adrenal of the dog secretes a substance which antagonizes the effects of aldosterone on the renal tubule[21] and that adrenalectomy removes this competitor. To explore the hypothesis that there may be antagonism between aldosterone and another adrenocortical steroid, a comparison has been made[419] between the effects on sodium and potassium excretion in Addisonian patients of intravenous infusions over 8 hours of aldosterone, aldosterone plus hydrocortisone and hydrocortisone alone. No antagonism was noted.

The duration of action of aldosterone is shorter than that of an equivalent dose of deoxycorticosterone, as judged by its effect on the excretion of sodium and potassium. Prunty and co-workers[274] found that 100 to 200 μg. of aldosterone given intramuscularly showed a maximum effect after 4 to 5 hours which lasted for 7 to 8 hours. The effects of 400 μg. lasted for 12 hours. The effect of 2 mg. of DL-aldosterone acetate given as an intravenous infusion over 8 hours lasted for only 2 to 6 hours after the infusion was stopped.[410] Probably because of its short-lived duration of action, the hormone appears to be more effective when given intramuscularly in oil, when it will be slowly liberated into the circulation, than when given intravenously as a single instantaneous injection.

A sodium diuresis and potassium anti-diuresis usually follow the sodium retention and potassium diuresis caused by the administration of aldos-

terone. The total 24-hour output of sodium may not be greatly different from control days because of this rapid reversal of effects [16]

Aldosterone given orally seems to be less effective in man than when given by the intramuscular or intravenous routes. Muller[336] noted no effect on electrolyte excretion from 500 µg. given orally whereas the same dose given intravenously to the same patient caused sodium, chloride and water retention. Others,[335] however, have found it effective when given by mouth. The short biological half-life of aldosterone (0.3 hr. (p. 44)) will account for the more prolonged effectiveness of the more slowly absorbed intramuscular injection.

ANTAGONISTS OF THE ACTION OF ALDOSTERONE ON THE RENAL TUBULE CELL

Synthetic steroids which antagonize the action of aldosterone on the renal tubule by competitive inhibition have been developed by Kagawa and colleagues.[231] Substances with this property are 3-(3-oxo-17 β-hydroxy-4-androsten-17 α-yl propionic acid γ-lactone (SC 5233) and its 19-nor analogue (SC 8109) (the 'spiro-lactones').

These substances modify the capacity of aldosterone (and of deoxy-corticosterone) to lower the sodium/potassium ratio in the urine of adrenalectomized rats[231] and antagonize the sodium-retaining effect of deoxycorticosterone acetate administered to a patient with Addison's disease,[236] resulting in a sodium diuresis despite the administration of 10 mg of deoxycorticosterone acetate daily to the patient.

In a dosage of 50 mg./hr., SC 8109 antagonized the effect of simultaneously infused aldosterone in two normal subjects.[407] The effect of the lactone was seen after a delay of 4 hours and lasted for 8 hours after the infusion was discontinued

The spiro-lactones have been shown to cause a sodium diuresis in normal subjects and in a patient with the nephrotic syndrome,[156] and a modest diuresis was produced in patients with cirrhosis [324a] Unlike other diuretics, they produce very little potassium loss

These substances appear to be effective only in the presence of aldosterone or other mineralocorticoid and presumably act by competitive inhibition. They may prove of great practical value as an investigative tool in assessing the role of aldosterone in human homeostasis and in the diagnosis of states of excessive aldosterone secretion.[413a] If derivatives can be devised of greater potency than those currently available, they may prove useful clinically in the management of oedematous states.

Effect of Aldosterone on Total Exchangeable Sodium and Potassium

Munro[344] reports that the administration of aldosterone in doses of 100 and 250 µg /day, caused no increase in total exchangeable sodium over a period of 7 days. The administration of 500 µg. of 9 α-fluorohydrocortisone, on the other hand, did so.

No change of total exchangeable sodium or potassium was found in patients with Addison's disease to whom aldosterone had been administered for a period of 7 days.

Effect of Aldosterone on Sodium and Potassium Secretion in Saliva, Sweat and Intestinal Juices

In addition to its effect on the handling of sodium and potassium by the renal tubule, aldosterone modifies the proportions of sodium and potassium secreted into the saliva and sweat and regulates the excretion of potassium and reabsorption of sodium through the mucosa of the gastrointestinal tract. The result of the administration of aldosterone, or of an increased secretion of aldosterone by the adrenal cortex, is a lowering of the sodium content of saliva, sweat or stools with a less pronounced effect on the potassium concentration of these excretions. In fact, the potassium content of the saliva of any particular individual is remarkably constant.[373] The overall effect of the steroid is to lower the sodium/potassium ratio of these fluids. Attempts have been made to utilize this effect as a simple measure of the level of circulating aldosterone. This hope, however, has not materialized in practice. While a low sodium to potassium ratio in saliva, sweat or stools is suggestive of increased aldosterone secretion it cannot be relied upon as a guide to the amount of circulating aldosterone.[447]

Reduction of the salivary sodium/potassium ratio resulting from the administration of aldosterone has been noted in patients with Addison's disease[118, 202, 440] A low sodium/potassium ratio in saliva and stools has been noted [illegible]

patients with the nephrotic syndrome

Effect on Tissue Electrolytes

The alteration of electrolyte composition of plasma, brain and muscle caused by the administration of 20 µg. of aldosterone daily for 3 days to intact mice has been studied *by Woodbury and Koch.*[334] *The plasma*

sodium concentration rose by 10 mEq./l. The intracellular sodium concentration of muscle and brain both became decreased and that of potassium increased. The ratio of extracellular to intracellular concentrations of sodium in muscle was greatly increased, that in brain slightly increased. The ratio of extracellular to intracellular concentrations of potassium in both muscle and brain was decreased. On these grounds the authors accept aldosterone as a promoter of ion transport across the cell membrane; 11-deoxycorticosterone acetate does not have this property when judged by these criteria.

Effect of Aldosterone on Water Excretion

Early experience with the administration of small doses of aldosterone (100 to 200 µg.) to man[225, 302, 374] suggested that this substance did not cause water retention, but with the administration of larger doses (400 to 1000 µg.) water retention with oedema formation occurred,[417, 418] as would be expected from a substance which causes sodium retention

Several investigators have noted that the administration of aldosterone to patients with Addison's disease in a dosage adequate to maintain electrolyte balance (up to 200 µg /day) does not correct the abnormal delay of excretion of a hypotonic water load found in this condition.[108, 225, 302, 374] Similar observations have been made in adrenalectomized animals.[108, 109, 192] The lowered glomerular filtration rate of adrenalectomized rats was not restored by the administration of aldosterone in doses of 0.2 µg per rat.[415] At much higher dosage levels (up to 120 µg. per rat), however, aldosterone proved equally as effective[295] as, or more effective[105] than, hydrocortisone, weight for weight, in rendering adrenalectomized rats resistant to water intoxication and in increasing glomerular filtration rate and renal plasma flow,[100] deoxycorticosterone was without activity in this respect.

The administration of aldosterone in high dosage (1000 µg. of DL-aldosterone acetate per day) to the adrenalectomized dog for a period of 3 weeks did not result in the diabetes-insipidus-like syndrome[193] such as appears when high doses of deoxycorticosterone are administered to this animal [110]

Evidence that aldosterone may inhibit the action of vasopressin on water reabsorption in the distal convoluted tubule of the kidney or that aldosterone and vasopressin may have opposing effects on water transport across the cells of the distal convoluted tubule, has been adduced by Levinsky, Davidson and Berliner.[244a] They noted that a substantial decrease in the maximum urine concentration, achieved during the administration of vasopressin and a forced water intake, occurred in dogs when

deoxycorticosterone was administered and when the animal was placed on a sodium-restricted diet. The effect of the latter is assumed to be that result of increased endogenous aldosterone production.

Effect of Aldosterone on Carbohydrate Metabolism

Reversion of glucose tolerance towards normal has been noted in man during the administration of aldosterone in maintenance doses to patients with Addison's disease.[illegible] Some impairment of carbohydrate tolerance appears to result from aldosterone administration in man[illegible] although this has not been noted by others.[illegible] McCullagh and co-workers[illegible] report three cases of Conn's syndrome due to adrenocortical adenomata; all showed a glucose tolerance test of diabetic type.

Schuler, Desaulles and Meier[illegible] found that aldosterone (in doses of 30, 100 and 300 μg. per mouse) was thirty times more effective (on a weight basis) than deoxycorticosterone, but only one-third as effective as [illegible] [illegible]

Effect of Aldosterone on Circulating Eosinophils

Depression of eosinophil count has been noted in man at a dosage of 1000 μg. per day.[illegible] A slight depression was noted by Kekwick and Pawan[illegible] in a patient with Addison's disease when given 100 μg. a day, but this has not been the experience of other investigators.[illegible]

In the Speirs and Meyer test[illegible] in adrenalectomized mice, aldosterone was one-half to one-third as effective as cortisone in causing reduction of the eosinophil count.[illegible]

Effect of Aldosterone on Corticotrophin Release by the Anterior Pituitary Gland

No suppressive effect on the release of corticotrophin by the pituitary gland has been noted in man as the result of the administration of aldosterone in a dose of 1 mg. a day[illegible] A patient with an aldosterone-producing adrenocortical adenoma who was excreting up to 120 μg. of aldosterone a day in the urine likewise showed normal 17-hydroxy and 17-ketosteroids in the urine, indicating a normal corticotrophin production.[illegible] Patients suffering from Conn's syndrome excrete normal amounts of corticosteroids in the urine.

Nevertheless, at high dosage (100 μg. per rat) aldosterone antagonized stress-invoked release of corticotrophin in the rat, as judged by adrenal

ascorbic-acid content.[110] In this test it had about one-third the potency of cortisone, and eight times that of deoxycorticosterone on a weight basis.

Effect of Aldosterone on Blood Pressure

No effect on the blood pressure was noted by Gross and colleagues in adrenalectomized, unilaterally nephrectomized rats given very large doses of D-aldosterone (40 µg. per rat) daily for 4 weeks.[194] A dose of deoxy-corticosterone acetate (1 mg/day) which had equivalent sodium-retaining activity produced hypertension and renal lesions. The same authors[194a] later found that a dose of 250 µg. of DL-aldosterone acetate daily for 28 days was without effect on the blood pressure of such rats, but the administration of 500 µg. of DL-aldosterone acetate per rat per day caused a rise of blood pressure as high as was obtained in those treated with deoxycorticosterone.[193, 194a] The administration of 0.5 to 1.0 µg. subcutaneously every 48 hours for as long as a year has been reported to cause hypertension in both intact and adrenalectomized rats.[146, 147] The simultaneous administration of reserpine reduced the hypertensive effect of aldosterone. Proteinuria was also exhibited by all the intact rats but by none of the adrenalectomized group. Oedema did not develop in any of the rats.

It is difficult to account for the discrepancy between the results of these two groups of workers since in the first instance the aldosterone was administered daily in oil intramuscularly and so could be expected to be effective over most, if not all, of the 24-hour period, whereas in the second instance the hormone was administered in alcohol subcutaneously and so would probably be effective for only a few hours of the 48-hour period between injections. The difference in result probably lies in the total length of the period of administration; in the first instance this was 29 days, in the latter 1 year. Little effect on blood pressure was noted during the first month and maximum effect was not reached until the fifth or sixth month.

Gross and Schmidt[194a] found that rabbits could not be made hypertensive when given aldosterone in doses of 1-2 mg. daily for 18 days, or when pellets of 75 mg were implanted subcutaneously.

Aldosterone, in doses adequate to maintain electrolyte balance, restores to normal and sustains the blood pressure in adrenalectomized dogs[274] and in patients with Addison's disease,[202, 274] without the production of hypertension

There is an obvious discrepancy, not yet resolved, between the very mild hypertensive action of aldosterone seen in the experimental animal

and in short-term experiments in man, and the severe hypertension encountered in cases of Conn's syndrome.

ALDOSTERONE AS REPLACEMENT THERAPY IN ADDISON'S DISEASE

Aldosterone provides adequate replacement therapy in cases of Addison's disease over the short periods during which it has been given to date; the longest is only of 6 days duration. In one particular case,[illegible] the administration of 1 mg /day (0.25 mg. every 6 hours by intramuscular injection) caused a weight gain of 3¾ lb. over the 6 days, with the development of oedema. There was sodium retention (the lowest urinary excretion was 37 mEq./day on an intake of 184 mEq /day) without any very pronounced effect on potassium excretion (highest urinary excretion was 105 mEq /day on an intake of 97 mEq /day). The depressive effect of aldosterone on the urinary sodium/potassium ratio in man is thus chiefly due to its effect on the tubular reabsorption of sodium.

Weight gain with the development of facial and ankle oedema has also [illegible] was noted.

Beck and co-workers[illegible] gave 600 µg. of aldosterone as a continuous intravenous infusion over 24 hours to an adrenalectomized patient and noted a weight gain of 2½ lb., which was lost in the succeeding 24 hours

Retention of sodium and chloride when aldosterone is administered to patients with Addison's disease has likewise been noted by Mach,[illegible] Kekwick,[illegible] Prunty,[illegible] Griboff,[illegible] Maclean[illegible] and Engel[illegible] and their colleagues Little effect on plasma levels of sodium or potassium has been reported, although a consistent fall of both plasma sodium and plasma potassium was seen during short-term intravenous infusions to patients with Addison's disease.[illegible] Engel and co-workers[illegible] comment that 300 µg. of DL-aldosterone acetate daily had a greater effect on the renal handling of sodium than had 25 mg. of cortisone acetate.

The minimum maintenance dose of aldosterone in cases of Addison's disease appears to be 150 to 200 µg. a day, or about 2.5 µg. per kg of body weight. The maintenance dose of D-aldosterone for adrenalectomized dogs is reported as 1.5 to 2.0 µg./kg./day.[illegible] Gross and Lichtlen[illegible] kept an adrenalectomized 11 kg dog in excellent condition for 3 months on a daily maintenance dose of 50 µg. of DL-aldosterone acetate. In this respect it is of interest that Mason in 1939[illegible] reported that he was able

to maintain an adrenalectomized dog alive by the administration of his most active preparation of 'amorphous fraction' in daily doses of 1 to 2 µg. per kg. Swingle and colleagues[473] found that smaller doses of D-aldosterone (1.5-3.1 µg. per dog daily), which were adequate to maintain a normal serum sodium concentration, led to hyperkalaemia. Swingle and co-workers later report that the administration of 20 mg. of DL-aldosterone monoacetate over 48 hours failed to relieve signs and symptoms of adrenal insufficiency produced in adrenalectomized dogs deprived of salt and steroid maintenance therapy.[484]

The maintenance dose of aldosterone given above restored the blood pressure of Addisonian patients to normal, but did not cause hypertension[392, 474] No mention of blood pressure is made in Thorn's[488] case given 1.0 mg. a day for 6 days.

Conflicting reports are given as to the efficacy of aldosterone in decreasing the pigmentation of Addison's disease. Lightening of skin pigmentation has been noted by some[335, 392, 382] but not by others,[386, 416, 474, 417, 488]

Aldosterone in high dosage (900 µg. over 36 hours) has not enough glucocorticoid activity to prevent the development of acute adrenal crises in adrenalectomized patients when cortisone maintenance therapy is withdrawn[308] It would therefore appear that it would be unsafe to use aldosterone as the sole maintenance therapy in cases of Addison's disease, although its lack of hypertensive action, compared with deoxycorticosterone, observed during long-term therapy in the experimental animal, would, if confirmed in man, make it a more desirable sodium-retaining adjunct to cortisone therapy than is deoxycorticosterone.

Gross and Lichtlen[192] noted that a state of adrenal insufficiency developed more quickly in adrenalectomized dogs following the withdrawal of maintenance therapy with aldosterone than after the withdrawal of deoxycorticosterone

Effect of Aldosterone on Granulation Tissue

Aldosterone, in doses of 300-5000 µg /kg subcutaneously in oil (i e. in 200 times the dosage required to produce an effect on the renal excretion of electrolytes) enhanced foreign-body granuloma formation in rats in response to the implantation of cotton-wool pellets[105, 143, 424] 11-deoxycorticosterone, in doses of 1 to 25 mg./kg. (fifty times that causing an effect on electrolytes) also had this action, whereas cortisone, in doses of 0 5 to 25 mg./kg , had an inhibiting effect, this is about the same dosage of cortisone as that effecting electrolyte excretion.

Anti-rheumatic Activity of Aldosterone

Aldosterone was found to have no anti-rheumatic activity in doses up to 1 mg /day.[216] This is perhaps not surprising as aldosterone does not possess a 17 alpha-hydroxyl group, believed to be essential for anti-rheumatic activity.[244a]

Maintenance of Lactation by Aldosterone

Cowie and Tyndal[11] observed that the administration of aldosterone in doses of 50 μg /day resulted in 'partial maintenance' of lactation in adrenalectomized rats. Complete maintenance of lactation was obtained with 100 μg. of chlorohydrocortisone acetate per rat per day, but not by 1 mg of hydrocortisone.

Effect of Aldosterone on Intestinal Motility

The peristaltic activity of the small intestine of rats was stimulated by aldosterone in doses of 0.1 to 0.5 μg, administered twice daily. Deoxycorticosterone and adrenal cortical extract in small doses also possessed this property, but not cortisone, hydrocortisone nor corticosterone in amounts comparable with those found in adrenal cortical extracts.[170a]

Effect of Aldosterone on Ion Transport Across the Red Cell Membrane

No effect of aldosterone, in concentrations up to 2.5 × 10^{-4} molar, on the rate of transport of potassium across the human red cell membrane could be demonstrated by Solomon, Gill and Gold.[457a] In such a system, the cardiac glucosides are strongly inhibitory. Glynn[173a] likewise failed to find any effect of aldosterone, in a concentration of 10^{-5} molar, on either sodium or potassium flux across the red cell envelope.

Effect of Aldosterone on Agglutination of Leucocytes

Stimulation of agglutination of leucocytes *in vitro* has been noted when DL-aldosterone monoacetate, 0.1 ug./ml, was added to the system. Cortisol, in a concentration of 2.0 μg./ml., inhibited the effect of aldosterone.[210a]

CHAPTER VI

THE CONTROL OF ALDOSTERONE SECRETION

At the present time it is not known how the liberation of aldosterone from the adrenal cortex is controlled. Certain facts have, however, been established. The first is that the adrenocorticotrophic hormone of the anterior pituitary gland is not a controlling factor of major importance. The second links the volume of some compartment of the extracellular fluid with the level of secretion of aldosterone. The third points to a *possible relationship between the rate of aldosterone secretion and the* concentration of potassium (but not of sodium) in the plasma.

The Influence of Trophic Hormones Secreted by the Pituitary on Aldosterone Secretion

(a) *Corticotrophin* (ACTH)

Even before the discovery of aldosterone, there had been pointers indicating that the secretion of the 'salt and water' hormone of the adrenal cortex was not directly under the control of adrenocorticotrophic hormone.

The evidence was derived in part from histological studies of changes in the adrenal cortex following hypophysectomy in animals Deane and Greep in 1946 for instance had noticed that the zona glomerulosa was unchanged or even hypertrophied in the hypophysectomized rat, in contrast with the atrophy of the other two *zones.*[97, 186] Similar observations in the hypophysectomized monkey were made by Knobil and colleagues.[241] Cytochemical staining reactions for ketosteroids remained positive in the zona glomerulosa, but became negative in the zona fasciculata.[185] In the dog, similar results were observed by Houssay and Sammartino.[221] Lane and de Bodo[280] also observed atrophy of all three zones following hypophysectomy in the dog but the atrophy of the zona glomerulosa was less conspicuous than that of the other zones. In the rhesus monkey, *the administration of corticotrophin resulted in hyper*plasia of the zonae fasciculata and reticularis, but not of the zona glomerulosa[241] Suppression of the pituitary in the monkey by exogenous cortisone resulted in a reduction of the width of the zona reticularis and zona fasciculata only, the zona glomerulosa being unaffected.[241] Similar effects of cortisone and cortisol have been observed in the dog.[186]

The administration of deoxycorticosterone acetate did not result in detectable alteration of the zona glomerulosa in the monkey,[311] but caused atrophy of this zone and depletion of lipid in its cells.[81, 166] Restriction of sodium intake, or an excessive potassium intake, led to widening of the zona glomerulosa with reduction of its content of lipid in rats,[81, 166, 266] and in dogs,[311] but in the latter the difference was not statistically significant. The hypertrophy of the cells of the zona glomerulosa which accompanied the restriction of sodium intake cannot, however, be prevented by the administration of deoxycorticosterone.[166] The hypophysectomized dogs with experimental ascites produced by inferior vena cava obstruction studied by Howell, Davis and Laqueur[311] showed normal zonae glomerulosae but atrophy of the zonae fasciculatae and reticulares.

These histological studies lead to the conclusion that the zona glomerulosa in animals, with the possible exception of the dog, is the zone responsible for the synthesis of aldosterone in these species, and that it is independent of pituitary control.

Suspicion that the regulation of mineralocorticoid differed from that of glucocorticoid had been voiced by Swann[471] in his *Physiological Review* written in 1940. He recognized that the adrenals were necessary for life, whereas the pituitary was not (except in the fowl) and concluded that the pituitary did not exert absolute control over the adrenal cortex and that hypophysectomized animals continued to secrete considerable amounts of sodium-retaining hormone.

Direct evidence that adrenocorticotrophic hormone is not a trophic hormone for aldosterone production is found in the reports of the existence of normal, or near normal, quantities of aldosterone in the urine of patients with panhypopituitarism. Luetscher[278] reported levels of 61 and 85 µg. deoxycorticosterone equivalent in two such patients; his five normal subjects had excretions ranging from 55 to 105 µg deoxycorticosterone equivalent per 24 hours. This observation has been confirmed. Venning, Dyrenfurth and Beck[499] estimated the sodium-retaining activity (presumed aldosterone) of the urine of five patients with hypopituitarism and of four hypophysectomized patients; in one case aldosterone could not be detected in the urine; in three other cases no aldosterone was present on some days but measurable levels were found on others In the other five cases the aldosterone excretion fell within the range of normal.

Measurement of aldosterone excretion in nine patients with hypopituitarism (receiving maintenance therapy of cortisone and thyroid) have also been made by Ross and colleagues.[411] The mean daily excretion in thirty determinations of the urine of nine patients receiving a normal sodium intake was 2.9 ± 0.4 µg /24 hr ; the mean (and S.D.) of the daily

excretion of normal subjects by the method used was 5.0±3.0 μg. The difference between means is significant ($p<0.01$). In only one of these nine patients was aldosterone absent from the urine when the patient was receiving a normal intake of sodium and in this case it appeared in the urine when the patient was placed on a low sodium diet; it also reappeared during the administration of corticotrophin. Luetscher[278] quotes the case of a patient with panhypopituitarism whose aldosterone excretion was at the lowest limit of normal when on a normal sodium intake, but whose aldosterone excretion increased sevenfold when the sodium intake was reduced to 10 mEq. a day.

Patients with longstanding idiopathic hypopituitarism have been submitted to sodium deprivation when receiving small maintenance doses of either cortisone or prednisone.[250a, 411] In both instances, the patient was able to conserve sodium adequately by reducing urinary excretion to levels equal to or below those of the sodium intake. In one instance[411] *this was accompanied by little rise in aldosterone excretion, in the other* patient[250a] aldosterone excretion increased from 12 to 25 μg./24 hr.

The original report by Farrell and Richards[142] of the occurrence of aldosterone in adrenal venous blood was based on data derived from hypophysectomized [illegible]

follows:[142]

	Hydrocortisone	*Cortisone*	*Aldosterone*
Sham-operated dogs	31 6	13 4	0 329
Hypophysectomized dogs	2 4	1 6	0 141
Hypophysectomized dog on corticotrophin	17.4	11 1	0 238

(All values expressed as μg /kg body wt /hr Aldosterone estimated by chromatography in two systems and bioassay)

The above table shows that the rate of secretion of aldosterone in adrenal vein blood from hypophysectomized dogs was 42% of that found in operated dogs, the comparable figure for hydrocortisone being only 7% and for corticosterone 12%. It was noted in this study that the rate of secretion of aldosterone was increased by the administration of corticotrophin, although to a much smaller extent than was that of the other steroids measured.

Farrell and co-workers[143] later reported that the aldosterone concentration (estimated by chromatography in two systems and bioassay) of adrenal vein blood bled out over a 4-hour period from three dogs 6

days after hypophysectomy was 66.5% of that obtained from three sham-operated animals; the corresponding percentages for other steroids were only 11.6 for corticosterone and 10 1 for hydrocortisone. The authors noted that the zona fasciculata of the hypophysectomized animals was reduced to half the thickness of that found in the untreated animal; there was no reduction in the thickness of the zona glomerulosa.

The ratio of sodium-retaining substance (presumed aldosterone) to corticosterone in adrenal vein blood of rats was found by Singer and Stack-Dunne to be altered by hypophysectomy from 0.3:1 to 3.65:1.[447] The amount of aldosterone (expressed as deoxycorticosterone-acetate equivalents) decreased from 45 μg./kg./hr. to 7.8 μg /kg./hr. in those experiments and the amount of corticosterone secreted fell simultaneously from 150 μg./kg /hr. to 2 μg./kg /hr.

The administration of corticotrophin to perfused glands, to hypophysectomized animals, to patients with panhypopituitarism and to normal subjects has yielded conflicting results.

The perfusion of calf adrenal glands with corticotrophin resulted in little change in the sodium-retaining activity of the effluent.[443] In only two of the eight experiments did the effluent produce a significant decrease of sodium excretion in the assay rats.

The production of aldosterone by rat adrenal glands incubated in Krebs-Ringer glucose solution, however, was found by Giroud and co-workers[171] to be stimulated by the introduction of corticotrophin into the medium. The sodium-retaining properties of the incubate was *estimated by bioassay of the crude chloroform extract or by ultra-violet* absorption. Some of the sodium retention observed in the bioassay may have been due to increased production of steroids other than aldosterone. In one set of experiments, in which sodium-retaining activity was measured by bioassay, the increase under the influence of corticotrophin was considerable, the 'aldosterone' produced being 0.4 μg./100 mg. of gland/hr. when no corticotrophin was present and ranging from 1.0 to 1 5 μg. when corticotrophin in doses of 1 to 100 milliunits was added to *the medium.*

In experiments by the same authors,[171] in which aldosterone was measured by ultra-violet absorption after separation on the Bush 'B5' chromatographic system, the increase was not so great, the mean of the control experiments when no corticotrophin was added being 0.52 μg./100 mg./hr. and the mean of the experiments when 100 or 200 milliunits of corticotrophin was added being 0.67 μg./100 mg /hr.

In man, Axelrad, Johnson and Luetscher[18] briefly stated in an abstract *that the output of sodium-retaining* hormone was *not* stimulated by

corticotrophin. Cope and Garcia-Llaurado[74] likewise report that corticotrophin did not increase the amount of sodium-retaining factor in the urine of a single case. Corticotrophin (100 units of gel injected intramuscularly every 12 hours) *has been administered by Liddle, Duncan* and Bartter[258] on fourteen occasions to normal subjects, some on a high, others on a low, sodium intake. The authors give no table of results but comment that on average the increase of 17-hydroxycorticoid excretion in the urine due to corticotrophin was tenfold, that of aldosterone only twofold. In this case aldosterone was measured by bioassay of crude methylene chloride extract. With prolonged corticotrophin therapy, aldostrone excretion was not maintained but fell to levels below those of the control periods. Withdrawal of corticotrophin after a short period of administration was followed by a fall of aldosterone excretion to very low levels, with accompanying sodium diuresis. The figures in this paper[258] show that corticotrophin caused a greater rise of aldosterone excretion when the patient was on a low sodium intake than when the same patient was on an intake of 100 mEq. of sodium.

Venning, Singer, Carballeira, Dyrenfurth, Beck and Giroud[500] assayed the urine of three normal subjects before and after the administration of corticotrophin and found no increase in sodium-retaining activity. Venning, Dyrenfurth and Beck[499] using an improved method of assay, later reported that they found no increase in aldosterone excretion in three normal male subjects when given corticotrophin. Three women with rheumatoid arthritis did show increases of up to a total of 10 µg/24 hr. when treated with corticotrophin. Continuation of corticotrophin therapy in one case was accompanied by a gradual fall to undetectable levels.

Further results of the effect of the administration of corticotrophin on aldosterone excretion in normal subjects are published by Muller, Riondel and Manning.[341] Eight patients were given corticotrophin, either intramuscularly or intravenously for 1, 2 or 3 days. In five of these patients a significant rise in the secretion of aldosterone (estimated by the physiochemical method of Neher and Wettstein) appears to have been obtained. A greater rise was seen in another normal subject given corticotrophin when on a dietary intake of 10 mEq. of sodium than occurred in another normal subject on a dietary intake of 110 mEq of sodium. A considerable rise of aldosterone excretion (about sevenfold) was noted by Beck and *colleagues[334] when two patients were given corticotrophin* immediately after an injection of vasopressin; in this experiment the increase found is not conclusive in supporting an effect of corticotrophin on aldosterone excretion, since a rise of aldosterone excretion occurs follow-

ing the withdrawal of vasopressin without the administration of corticotrophin.

The effect of intravenous infusions of corticotrophin on aldosterone excretion was investigated on twenty-one occasions by Hernando, Crabbé Ross, Reddy, Renold, Nelson and Thorn.[111] An increased excretion of aldosterone during corticotrophin administration was found in three patients with Cushing's syndrome and in subjects on a sodium-restricted

tion are available for 2 control days, 2 days on which corticotrophin was infused, and the 2 succeeding days in six patients; the mean for these periods are respectively 9, 11 and 3 µg./24 hr. The authors comment that the administration of corticotrophin does not lead to any conspicuous augmentation of aldosterone excretion except in circumstances where the patient's adrenals were hyperactive, as in Cushing's syndrome, or unless the subject was maintained in a situation (e.g. on a low sodium intake) which itself results in an increased aldosterone excretion ('secondary hyperaldosteronism').

Corticotrophin gel was administered for a period of 14 days to a normal subject in doses of 40 units a day intramuscularly, following a control period of 12 days.[411] The sodium and potassium intakes were constant throughout (Na = 69 mEq., K = 77 mEq /day. The mean aldosterone excretion during the control period was 4 2 µg /24 hr.; during the period *of corticotrophin administration it was 9.7 µg./24 hr. There is no evidence* of a reduced response with continuation of corticotrophin administration.

Tronchetti, Mucio and Romanelli[4440] found that the administration of corticotrophin (two injections of 40 units at 12-hour intervals) to normal subjects increased aldosterone excretion by 90% within the first 24 hours, followed by a fall to subnormal levels by 72 hours. Thyrotrophin was also found by these authors to increase aldosterone excretion by 55%.

Simpson and Tait[440] analysed human peripheral blood for aldosterone *before and 2½ hours after the administration of corticotrophin* (25 units of gel intramuscularly) No increase in activity was found, as determined by the lowering of the sodium/potassium of the urine of adrenalectomized rats The plasma hydrocortisone level increased threefold during this period

The administration of prednisone, cortisone or cortisol, in doses adequate to suppress anterior pituitary function, did not result in a fall of aldosterone excretion in human subjects[115, 159, 400] nor in a decreased rate *of secretion of aldosterone into adrenal vein blood in dogs.*[115]

The excretion of aldosterone in the urine of patients undergoing hypophysectomy has been studied by Llaurado[248] and by Ross, van't Hoff, Crabbé and Thorn.[411] Llaurado[248] found that aldosterone persisted in the urine after hypophysectomy in man, but the amount excreted did not show the substantial increase found by Llaurado[248] following other surgical procedures. Ross, van't Hoff, Crabbé and Thorn[411] do not agree with these findings. They measured the excretion of aldosterone in the urine of four patients subjected to hypophysectomy for the palliation of carcinoma of the breast. All four patients developed diabetes insipidus following hypophysectomy and all showed a substantial rise of aldosterone excretion during the period when their diabetes insipidus was not controlled by posterior pituitary extract. A value of 106 µg./24 hr. was observed in one patient who was pregnant. In the other patients values of up to 25 µg./24 hr. were recorded.

One of these hypophysectomized patients was placed on a restricted sodium intake (13 mEq./day) 4 months after hypophysectomy, at a period when her polyuria was controlled by posterior pituitary extract. After 5 days her aldosterone excretion rose to 21 µg./24 hr. Such a good response to sodium restriction was not found in patients who have had hypopituitarism for some years,[325, 411] but has been reported by Maclean [303] in patients within a year of hypophysectomy.

The following histological observations also suggest that the pituitary gland participates in some manner in the regulation of aldosterone secretion Selye[441a] noted that the intraperitoneal injection of hypertonic saline to rats gave rise to certain characteristic changes in the zona glomerulosa of the adrenal gland These changes only occur in the presence of the hypophysis and are absent in hypophysectomized rats.[447a] The implantation of freshly removed rats' pituitaries into the hypophysectomized animal restores the effect The histological changes in the adrenal are similar to those which occur during states of increased aldosterone secretion[311] and suggest that the administration of hypertonic solutions of sodium chloride releases some humoral substance from the pituitary which alters the histological appearance of the zona glomerulosa

It may be concluded from these extensive studies that the adrenocorticotrophic hormone of the anterior pituitary gland is not a major factor in the regulation of the secretion of aldosterone. Corticotrophin, however, is not entirely devoid of effect on the secretion of aldosterone as judged by observations on the urinary excretion of aldosterone, since under certain conditions, notably in patients on a low sodium intake, considerable augmentation of aldosterone excretion may occur during the administration of corticotrophin. Patients with longstanding hypopituitarism

excrete quantities of aldosterone significantly below normal when on an unrestricted sodium intake; the response of their aldosterone excretion to the stimulus of sodium restriction is also reduced. However, immediately following hypophysectomy they are capable of excreting large quantities of aldosterone during the period of polyuria due to diabetes insipidus, and 4 months after hypophysectomy can respond to sodium restriction by a considerable increase in aldosterone excretion. It is evident that the capacity of the adrenal cortex to respond to suitable stimuli by an increased secretion (excretion) of aldosterone falls off very slowly following loss of anterior pituitary function. Whether the stimulus provided by corticotrophin acts directly on the synthesis or release of aldosterone, or indirectly by increasing the size of the adrenal gland, is at present not known.

Some of the confusion evident in the results of studies of the influence of corticotrophin in the secretion of aldosterone may be due to contamination of corticotrophin with 'aldosterone-stimulating hormone' during its manufacture, or to the presence of varying amounts of different corticotrophin fractions in the preparations used. Farrell and colleagues[148] have assayed the effect of two corticotrophin fractions prepared by Bell[174] on aldosterone secretion into adrenal venous blood of hypophysectomized dogs They found that the infusion of δ_1-corticotrophin resulted [illegible]

(b) *Effect of growth hormone*

A stimulating effect of growth hormone (somatotrophin) on the excretion of sodium-retaining factor extracted from unhydrolysed urine of normal subjects was reported by Venning and co-workers in 1955.[363] The growth hormone used was prepared by the method of Raben and Westermeyer.[377] Material prepared by the method of Li, however, was later shown to be without effect on the excretion of sodium-retaining factor extracted from hydrolysed urine and purified by a single chromatographic procedure. A similar lack of effect on aldosterone excretion (measured by a physicochemical method) was reported in a single subject by Hernando and co-workers.[319] In hypophysectomized rats growth hormone was found to be ineffective in modifying the rate of secretion of aldosterone into adrenal vein blood[447]

In vitro studies have likewise shown that growth hormone had no effect on the rate of secretion of aldosterone by the prefused adrenal

gland.[403] A similar lack of effect has been noted with incubated rat adrenal glands.[271]

The experiments reported above were carried out with growth hormone obtained from animal sources (beef or hog). In 1957 Beck, McGarry, Dyrenfurth and Venning[37] reported a striking increase in aldosterone excretion in a pituitary dwarf given human growth hormone prepared by the method of Raben and Westermeyer. The daily excretion of aldosterone increased from 2.8 to 20.2 μg./24 hr. The administration of monkey growth hormone to the same patient resulted in a more modest response — from 3.2 to 12 μg./24 hr. No effect was observed on the excretion of 17-hydroxycorticoids.[500] It has subsequently been stated that Venning and co-workers have found the administration of human-growth hormone to a total of three patients has resulted in an increased aldosterone excretion in all three.[500] The possibility that the growth hormone used may have been contaminated with 'aldosterone-stimulating hormone' must be borne in mind in assessing the significance of these results.

(c) Effect of the administration of gonadotrophin on aldosterone excretion

A single observation is reported by Thorn and co-workers.[447] No change in aldosterone excretion during the administration of chorionic gonadotrophin was seen. Tronchetti, Mucio and Romanelli[440b] likewise found no change in the excretion of sodium-retaining factor following the administration of gonadotrophin.

THE EFFECT OF CHANGES OF ELECTROLYTE CONCENTRATION IN THE PLASMA

(a) *The plasma sodium concentration*

Observations on the level of the plasma or serum sodium concentration in relation to the amount of aldosterone excreted in the urine suggest that there is little relationship between the two.

Hyponatraemia occurring in hypopituitarism or during the administration of vasopressin is not associated with aldosterone levels outside the normal range.[38, 411] In one case of hypopituitarism the serum sodium concentration fell to 111 mEq./l., without any change in aldosterone excretion.[411] Hyponatraemia due to haemodilution resulting from the administration of vasopressin and water is associated with a decreased level of aldosterone excretion.[414]

The restriction of fluid intake in a normal subject receiving a high intake of sodium resulted in hypernatraemia with reduced vascular volume and is associated with an increased excretion of aldosterone.[414] On the other

hand, the administration of hypertonic saline to a normal subject, resulting in hypernatraemia with increased vascular volume, was in this case associated with a reduced excretion of aldosterone.

It can only be concluded from the lack of correlation between the levels of sodium in the blood and of aldosterone in the urine that the concentration of the plasma sodium has little or no direct influence on the level of secretion or excretion of aldosterone.

(b) *The plasma potassium concentration*

Several observations suggest that a relationship may exist between the serum potassium concentration and aldosterone secretion or excretion.

In the experimental animal, Singer and Stack-Dunne[illegible] found that rats made potassium deficient by dietary means secreted very low levels of aldosterone (measured by chromatography in a single system and bioassay) into adrenal vein blood — about one-twentieth of normal. The level of corticosterone secretion was unaffected.

In an histological approach, Stoerk, Knowlton and Loeb[illegible] measured the width of the zona glomerulosa in serial sections of rat adrenal glands and so obtained an estimate of the weight of this zone. Using dietary manipulation to vary the level of the serum potassium they found a direct relationship between the weight of the glomerulosa and the level of the serum potassium. Deane, Shaw and Greep[illegible] likewise noted hypertrophy of the zona glomerulosa in rats when potassium intake was increased.

In dogs, Laragh and Stoerk[illegible] could detect no sodium-retaining activity in the urine when the animals were fed a sodium-restricted diet or subjected to peritoneal dialysis unless a potassium-chloride supplement was given at the same time, resulting in an elevated serum potassium concentration. These authors concluded that the administration of potassium has little influence on aldosterone secretion unless a significant hyperkalaemia is caused

In a perfused calf adrenal preparation, Rosenfeld, Rosemberg, Ungar and Dorfman[illegible] found that increasing the proportion of potassium to sodium in the perfusing fluid increased the rate of secretion of aldosterone. [illegible]

a [illegible]

s [illegible]

by quartered rat adrenal glands incubated in Krebs-Ringer bicarbonate solu[illegible]

flu[illegible]

I [illegible]

terone in a patient with pyloric stenosis who was hypokalaemic, but other factors including dehydration must have been present in this case.

Several authors have reported alterations in aldosterone excretion as the result of changes in the potassium and sodium content of the diet. Thus, Luetscher and Curtis[384] contrast the excretion of sodium-retaining hormone in a normal subject when on a low sodium diet (Na intake = 9 mEq., K intake = 70 mEq.) and when on a high potassium diet (Na intake = 120 mEq., K intake = 200 mEq.) and note the elevated excretion in both circumstances; the increase was greater when the intake of sodium was low than when potassium was high. An elevation of aldosterone excretion consequent upon potassium loading has also been noticed by Falbriard, Muller, Neher and Mach[136] A three or fourfold increase in these circumstances was found by Nowaczynski, Koiw and Genest.[357] On the other hand, little difference in aldosterone excretion was noted by Hernando and co-workers[215] when a normal subject was placed on a high potassium intake. A sodium diuresis and loss of weight occurred in these experiments Liddle, Bartter, Duncan, Barber and Delea[360] comment that the administration of potassium chloride to normal subjects resulted in an elevation of aldosterone excretion only in those subjects in whom it induced a weight loss.

Potassium loading results in a sodium diuresis and loss of weight and so presumably in loss of extracellular fluid. A potassium load may therefore merely be another way of influencing a volume receptor. That this is not the whole story is suggested by the experiment of Bartter[22] who replaced the sodium lost during potassium loading so that the body weight remained constant; an elevation of aldosterone excretion still occurred during the period of potassium loading. In this connection it would be expected that sodium retention, rather than sodium diuresis, would ensue if potassium loading really gave rise to a significant increase of aldosterone secretion.

Restriction of potassium intake in a normal subject on a normal sodium intake reduces the excretion of aldosterone.[26] Expansion of the extracellular fluid is said to occur in potassium depletion[41, 334] so that restriction of potassium intake may again be a method of altering extracellular fluid volume. Restriction of potassium intake in a normal subject during a period of dietary restriction of sodium lowers the level of aldosterone excretion, thus antagonizing the effects of sodium restriction.[22] Nevertheless elevation of aldosterone excretion in response to the low sodium diet still occurs if the intakes of both sodium and potassium are restricted although the response is lower than when sodium alone is restricted.[333]

The expansion of body fluid caused by the administration of vasopressin and water, if accompanied by the simultaneous administration of a high potassium intake, results in an increased, rather than the usual decreased, excretion of aldosterone.[11]

The elevation of aldosterone excretion which occurs during potassium loading is less pronounced than that which occurs during restriction of sodium intake.[344]

Although many of the experiments quoted above suggest that an increased potassium intake, or a rise of the serum potassium concentration, results directly or indirectly in an increase of aldosterone excretion, direct measurement of the rate of secretion of aldosterone into adrenal vein blood of dogs by Rosnagle and Farrell[144] failed to reveal any significant change of aldosterone secretion when the dogs received an increased dietary potassium intake plus an intravenous infusion of potassium chloride for 90 minutes before the collection of the adrenal vein blood.

It is thus not yet clear whether an alteration of potassium balance by itself is capable of modifying the rate of secretion of aldosterone, or whether it is the volume consequent upon alteration of potassium balance which is the responsible stimulus.

Effect of Plasma Osmolarity on the Excretion of Aldosterone

The osmotic pressure of the plasma is largely due to its sodium-chloride content. The failure of changes of the plasma sodium concentration to influence the amount of aldosterone excreted in the urine would make it appear that the osmolarity of the plasma is not a controlling factor in aldosterone production. Measurements of the osmolarity of the plasma and of aldosterone excretion in various states of secondary hyperaldosteronism by Wolff and co-workers bear out this conclusion.[329, 330, 331] Aldosterone excretion is diminished whenever the extracellular volume is expanded by either water, which decreases plasma tonicity, by normal saline which leaves plasma tonicity unchanged or by hypertonic saline which increases plasma tonicity. Conversely aldosterone excretion increases whenever the extracellular volume is contracted either by dehydration which increases plasma tonicity, by diuretics which leave plasma tonicity unchanged or by the withdrawal of vasopressin in a patient with diabetes insipidus, which increases plasma tonicity

Effect on Aldosterone Excretion of Changes in Volume of Body-water Compartments

The identification of the 'sodium-retaining factor' present in urine as

aldosterone and the demonstration, mainly by Luetscher and co-workers, of increased amounts of aldosterone in the urine of patients with congestive heart failure, the nephrotic syndrome and in hepatic cirrhosis – all states characterized by oedema, that is, by an excess of extracellular fluid – suggest that there might be a link between an expanded extracellular fluid volume and an elevated secretion of aldosterone. However, it was difficult to implicate an expanded extracellular volume as the cause of increased aldosterone secretion when the only known experimental methods of raising the level of aldosterone excretion in the urine involved methods of reducing the volume of body water. Procedures to achieve this may be either direct, e.g. by phlebotomy, or indirect, e.g. by placing the patient on a low sodium diet, by excessive sweating or by the administration of diuretics. This apparent paradox can be resolved by postulating that the regulating volume is not that of total extracellular volume, but is confined to the volume of the vascular compartment or to some section of the vascular compartment. The experimental evidence leading to this conclusion will be surveyed.

The increase of aldosterone excretion which occurs in normal subjects when dietary intake of sodium is restricted, originally observed by Luetscher and colleagues,[331] has been amply confirmed.[12, 81, 215, 333] No concurrent increase in excretion of 17-hydroxycorticoids or 17-ketosteroids occurs,[84] neither is there any measurable change in glomerular filtration rate as measured by the renal clearance of inulin or creatinine.[331] Luetscher and Axelrad[331] noted a slight fall of serum sodium and a 'moderate' decrease of body weight (1·7 kg in the one case in which body weight is given).

More extensive data about weight loss and aldosterone response during dietary restriction of sodium have been provided by Crabbé, Ross and Thorn[82] and are discussed in Chapter XI. Estimation of aldosterone secretion rate in man by means of tritiated aldosterone confirms that an increased amount of aldosterone is secreted during periods of sodium withdrawal.[174, illegible]

In animals, the aldosterone content of dog adrenal vein blood is almost doubled during the administration of a low salt diet.[405] In the rat, aldosterone secretion, but not that of corticosterone, is increased when the animals are fed a sodium-deficient diet.[138]

Sodium deprivation which does not result in weight loss does not result in an increased aldosterone excretion.[illegible] Restoration of fluid equivalent to the weight loss returns the level of aldosterone excretion to normal regardless as to whether the fluid used to restore the original fluid loss contains sodium or not.[illegible] The factor which is effective in modifying

the secretion of aldosterone during the administration of a low sodium intake is therefore considered to be a reduction of body water content.

A similar increase of aldosterone excretion follows the administration of diuretics such as mercurials[illegible] or acetazoleamide (Diamox)[illegible] without change of plasma tonicity. The administration of potassium chloride concurrently with acetazoleamide resulted in a greater increase in aldosterone excretion and a greater sodium diuresis and weight loss than with either acetazoleamide or potassium chloride alone.[illegible] In all cases the administration of diuretics resulted in the loss of water, sodium and potassium. The administration of potassium alone *likewise results in a* diuresis of sodium and water, with weight loss which is accompanied by an increase in aldosterone excretion.[illegible]

Extrarenal loss of water and sodium induced by sweating as the result of actively engaging in strenuous exercise[illegible] or passively by sitting in a Finnish steam bath[illegible] or by exposure to hot environments,[illegible] pastimes which are associated with excessive sweating and loss of water and sodium through the skin accompanied by renal retention of sodium and water, resulted in an increase in aldosterone excretion. These circumstances led to a greater loss of water than of sodium, with resulting plasma hypertonicity and hypernatraemia. Prevention of the fluid deficit by the oral administration of water in one such study led to only insignificant fluctuations of aldosterone excretion being observed.[illegible] This type of experiment enables a dissociation to be made between loss of water with and without concomitant loss of sodium, so that an evaluation of the importance of these two variables can be made. A similar dissociation is also effected by the administration of water during the anti-diuresis which follows the administration of anti-diuretic hormone (vasopressin) and during the diuresis which follows its withdrawal. During the period of administration of vasopressin there is water retention, haemodilution with hyponatraemia and weight gain but an increased loss of sodium in the urine is observed compared with the control period,[illegible] aldosterone excretion is reduced during this period.[illegible] If the administration of vasopressin is now discontinued there is a diuresis of water and loss of body weight, together with the return of plasma sodium concentration to normal, but retention of sodium in the urine is observed associated with an increased excretion of aldosterone *It is important to note that the* level of aldosterone excretion does not rise during the period of sodium loss and hyponatraemia caused by vasopressin administration, indicating that the level of plasma sodium does not appear to be a factor influencing the level of aldosterone secretion, a conclusion also drawn from other

observations on the lack of correlation between the level of the plasma sodium concentration and the excretion of aldosterone (p. 68).

Restriction of fluid intake in normal subjects (and in one patient with hypopituitarism) receiving diets containing a moderate amount of sodium results in a contracted extracellular volume and hypernatraemia; a similar situation results from the withdrawal of vasopressin in a patient with diabetes insipidus.[11, 111] In all cases studied (including the patient with hypopituitarism) there was an increased excretion of aldosterone during the period of dehydration.

The more rapid the weight loss during the period of dehydration, the greater was the elevation of aldosterone excretion. In one case, where the weight loss was about 0.5 kg. a day, the elevation of aldosterone excretion was very slight (from 3 to 6 µg./24 hr.). In the same patient the administration of mercurial diuretics, which caused a weight loss of 1.5 kg. in one day, was associated with an elevation of aldosterone excretion of from 4 to 26 µg./24 hr.[11]

The administration of hypertonic saline to normal subjects on a low sodium diet, thereby re-expanding the extracellular volume, resulted in a fall to normal of the elevated aldosterone excretion.[111]

Indications that the controlling volume is related to the volume of the vascular bed is given by the following observations. The intravenous administration of albumin to a patient with oedema due to idiopathic hypoproteinaemia, who was excreting very high amounts of aldosterone, caused a prompt fall of aldosterone excretion associated with a sodium diuresis and weight loss.[14] In this case the plasma volume was expanded by the albumin and the extravascular extracellular volume contracted by the subsequent diuresis. The same investigator[14] removed 700 ml. of blood from a normal subject (maintained on a low sodium diet so that he had an elevated aldosterone output) but replaced the sodium with 350 ml of physiological saline; the excretion of aldosterone increased from 60 to 140 µg./24 hr (judged by Fig. 4 of the paper), but fell to its previous level when the red cells were returned together with salt-poor albumin to restore the blood volume to its previous dimensions. Fine, Meiselas and Auerbach[144] similarly report an increased excretion of sodium-retaining hormone following phlebotomy of 450 to 670 ml. of blood in man.

Progressive acute blood loss in the dog led to an increased rate of secretion of aldosterone in (pooled) adrenal venous blood.[111] This increased secretion could be delayed by replacement of the blood loss by 6% dextran in normal saline, but not by a solution of gelatin in normal saline which escaped from the vascular bed much more quickly. In the

third hour of bleeding the rate of secretion of aldosterone rose despite replacement with dextran. Chronic haemorrhage in the dog has likewise been noted to result in increased urinary aldosterone excretion;[illegible] this may be the explanation for the reduced urinary excretion of sodium noted during both acute and chronic haemorrhage in man.[illegible] These experiments are not conclusive in narrowing down the probability that the regulatory mechanism responds solely to changes in circulating blood volume since there was a loss of 42 mEq. of sodium in the blood removed; this loss was not compensated for by raising the sodium intake. An increase of aldosterone excretion of the same magnitude was obtained by these authors in untreated dogs by reducing the sodium intake to the same net amount (18 mEq /day).[illegible]

The relationship between the plasma volume and the rate of secretion of aldosterone may not be quite as straightforward as appears from the experimental data quoted above. Wolff[illegible] has pointed out that following phlebotomy the plasma volume is restored (within 12 to 24 hours) *whereas the excretion of aldosterone remained elevated for 72 to 100 hours* following phlebotomy. Nevertheless, the observed facts are all consistent with the hypothesis that the 'salt-retaining hormone responds to a reduction of the fullness of the bloodstream, however and wherever this may be registered'* to quote Peters.[illegible]

The possibility of the existence of an intracranial structure concerned with the regulation of aldosterone secretion has been raised by Rauschkolb and Farrell.[illegible] They found that neither transection of the cord at the level of the third cervical vertebra nor decortication influenced the rate of secretion of aldosterone into adrenal vein blood. Decerebration at mid-collicular level and decapitation both reduced the rate of secretion of aldosterone to about 30% of normal. The conclusion was that aldosterone secretion is controlled by a regulating factor released by some structure situated in the diencephalon. Destruction of the reticular formation in the caudal diencephalon and mesencephalon has been reported to result in a significant reduction of aldosterone into adrenal vein blood in the cat.[illegible] A lesion in the rostral half of the diencephalon, on the other hand, resulted in an increased rate of secretion of aldosterone. In this context it is of interest to recall that Peters[illegible] considered that the controller of the rate of [illegible] under certain conditions of temperature, caused a fivefold increase in the

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Progressive acute blood loss in the dog led to an increased rate of secretion of aldosterone in (pooled) adrenal venous blood[illegible] Thus increased secretion could be delayed by replacement of the blood loss by 6% dextran in normal saline, but not by a solution of gelatin in normal saline which escaped from the vascular bed much more quickly. In the

The afferent pathway from receptors in the atria to the centre in the diencephalon has not been defined. Fibres running in the vagus nerve which respond to stretching have been described originating from the atria and the roots of the great veins.[444] Section of the vagus, however, did not prevent the increased secretion of aldosterone that followed constriction of the inferior vena cava (which reduces filling of the right atrium), but did prevent the fall in secretion which followed release of [illegible] of aldosterone take another pathway. Alternatively, the receptor responsible for initiating impulses which result in increased secretion of aldosterone may be situated in an entirely different site, such as on the arterial circulation.

Aldosterone appears to be unique among the steroids secreted by the adrenal cortex in that its rate of secretion is not primarily dependent upon anterior pituitary control. Its principal controlling mechanism appears to be closely related to the volume of the vascular bed and there is some experimental indication that the receptors on the afferent side of the reflux are situated in the right atrium, and perhaps also somewhere on the arterial side of the circulation. Afferent impulses travel from this receptor, or receptors, to a centre situated in the mesencephalon, which in turn influences an effector organ, situated perhaps in the diencephalon, which secretes the humoral factor, discussed in the next section, which influences the rate of secretion of aldosterone by the zona glomerulosa of the adrenal cortex.

'Aldosterone-stimulating Hormone'

The postulation of a centre controlling the secretion of aldosterone which is situated outside the adrenal cortex, possibly in the diencephalon, implies the existence of a connecting link between the centre and the adrenal cortex. There is some evidence to indicate that this is humoral rather than nervous, since Fleming and Farrell[111] found that the rate of secretion of aldosterone by the transplanted dog adrenal was higher, rather than lower, than that of control dogs with normally innervated adrenal glands.

Orti, Ralli, Laken and Dumm[111] have studied the effect of injections of the urine of adrenalectomized rats upon the sodium excretion of intact rats under certain circumstances. When adrenalectomized rats were maintained on a salt-free diet, the intraperitoneal injection of 1 ml. of their urine into intact rats caused a decreased urinary excretion of sodium.

rate of secretion of aldosterone into adrenal vein blood, in experiments on decerebrate dogs.

Farrell's observations localize the position of centres concerned with the reflex regulation of aldosterone secretion to an area in the diencephalon. The receptor on the afferent side of the reflex arc appears to be an organ sensitive to some stimulus connected with the circulatory system. The site of this receptor is probably not within the skull. Although it has been observed that changes in posture and compression of the neck resulted in alterations in sodium excretion,[illegible] Fishman[illegible] failed to influence the renal excretion of electrolyte by changes in intracranial venous pressure or in the volume of the cerebrospinal fluid.

Location of the hypothetical receptor on the arterial side of the circulation has been suggested. The full report of Bartter's work on receptors in this site is not yet available.[illegible] One difficulty with placing the receptors somewhere on the arterial circulation is the observation that sodium retention occurs in cases of high output failure, where the cardiac output is increased, as well as in low output failure, where the cardiac output is decreased.

Many authors have suggested that a receptor influencing electrolyte excretion by the kidneys is situated in some part of the vascular bed within the thorax Barger and colleagues[illegible,b] noted that experimental tricuspid stenosis in the dog led to diminished excretion of sodium without measurable change in glomerular filtration rate. The addition of pulmonary stenosis to the tricuspid lesion results in frank congestive failure, with almost quantitative sodium retention. Henry and colleagues[illegible,b] have adduced evidence for the location of receptors in the cardiac atria influencing both sodium and water excretion. The observations of Love and colleagues[illegible] suggest that these receptors respond to pulsatile pressure rather than to simple stretching.

There is circumstantial evidence that aldosterone is concerned with the alteration of renal handling of sodium noted in the above studies of atrial volume receptors. Observations that stretching of the right atrium in the dog modifies the rate of secretion of aldosterone have been made by Anderson, McCally and Farrell.[illegible] These investigators stretched the atria by means of sutures attached from the atrial appendages to the chest wall. When the left atrium was stretched, the rate of secretion of aldosterone into adrenal venous blood over a two-hour period was not significantly different from sham-operated controls. Aldosterone secretion, however, was significantly reduced when the right atrium was stretched; the mean of the sham-operated dogs was 43 3 µg /100 kg. body wt./hr., that of the group with right atrial stretching was 23 8 µg.

CONN'S SYNDROME

DEFINITION

A CLINICAL syndrome attributed to the secretion by the adrenal cortex of excessive amounts of aldosterone was described in 1955 by Conn[47, 478, 79] under the title of 'Primary Aldosteronism'. The principal features of this syndrome are the recurrence of episodes of muscular weakness associated with hypokalaemia, hypochloraemia and alkalosis, hypertension, polyuria which is resistant to vasopressin and in some cases tetany in the presence of normal serum calcium and phosphorus concentrations. The absence of oedema is a noteworthy feature of this syndrome.

Since the original description by Conn, numerous further cases, proven or probable, have been described. Details of well-documented cases are presented in Table V.

The majority of the reported cases have been due to adrenocortical adenoma. One has been due to an adrenocortical carcinoma[134] and five to bilateral hyperplasia of the adrenal cortex.[19, 117b, 120, 124a, 423, 424] The majority of cases have been in women. Four cases have been reported in children or adolescents aged 9, 13, 13 and 17 years.[20, 110, 143, 424] The pathology of the adrenals was reported as normal in two of these children,[20, 110] in the other two bilateral hyperplasia was present.[143, 424] An adrenocortical adenoma has yet to be reported as the cause of Conn's syndrome in children.

SIGNS AND SYMPTOMS

Although Milne, Muehrcke and Aird[317] have described an asymptomatic case, the presenting complaint in the majority of well-documented cases described to date has been the recurrence of episodes of weakness lasting from a few hours to 3 weeks; in some cases there was complete paralysis of limbs. This symptom can be ascribed to the hypokalaemia and depletion of total body potassium. Chalmers and his colleagues[69] found a total exchangeable potassium of only 29·6 mEq./kg. body weight on one occasion in their patient, the mean and S.D. for normal females by the method used was 39·0±5·4. A patient with this syndrome, however, can have a very low serum potassium concentration for long periods of time with only occasional attacks of paresis or paralysis,[124, 421] so that some other factor seems to be present to modify the effects of

On the other hand, when the adrenalectomized rats were maintained on a 1% saline drink, their urine did not cause sodium retention in the recipient intact rats. This urinary substance did not cause sodium retention in adrenalectomized rats. Aldosterone excretion in the faeces was present in greater amounts in intact rats receiving urine from adrenalectomized rats on a sodium-restricted diet than in those receiving urine from adrenalectomized rats who were allowed to drink 1% saline. The conclusion from these observations was that some substance was present in the urine of adrenalectomized rats deprived of salt which stimulated the adrenals of intact rats to secrete aldosterone.

These experiments have been repeated by Ross, Miller and McLean.[444] A substance was found to be present in the urine of adrenalectomized rats deprived of sodium in both food and drinking water, which lowered the sodium/potassium ratio in the urine of intact rats, but not of adrenalectomized rats, when injected intraperitoneally. The administration of corticotrophin did not produce this type of response. It has yet to be proved that aldosterone is the substance responsible for the fall in the sodium/potassium ratio in the urine of the recipient rats, but chromatography of chloroform extracts of this urine on the toluene-propylene-glycol and Bush 'B5' systems, followed by bioassay, showed that sodium-retaining properties were present in the position occupied by aldosterone in greater quantities in the urine of recipient rats receiving urine from sodium-deprived donors than in those receiving urine from donor rats on a normal sodium intake.[444]

'Aldosterone-stimulating hormone' could not be demonstrated in the urine of a normal subject on a low sodium diet (after extraction of aldosterone by chloroform) nor in the urine of an adrenalectomized patient deprived of steroid maintenance therapy.[444] This may mean that the hormone is species specific.

Farrell[137] has suggested the name 'glomerulotropic hormone', or 'glomerulotropin' for this hormone, instead of 'aldosterone-stimulating hormone', postulating that it acts on a particular type of cell, those of the zona glomerulosa, rather than regulating particular enzymic processes within the cell, such as steroid 18-hydroxylation. Since all the precursors of aldosterone appear to be present within the cells of the zona fasciculata, and yet little aldosterone is made there, the trophic hormone must either be cell specific or the zona fasciculata deficient in the requisite enzyme, steroid 18-hydroxylase.

hypokalaemia. Black and Milne[41] observed that paralysis due to hypokalaemia is more frequent in the presence of metabolic acidosis than if the arterial pH is high; the alkalosis of Conn's syndrome probably protects the patient to some extent from paralysis.

All but one case had hypertension; the exception,[124] in whom the diagnosis of Conn's syndrome is presumptive only, was in fact hypotensive, with a blood pressure *of 70/50 which fell on standing to 60/30.* One case had convulsive seizures.[220] Two cases[219, 401] had malignant hypertension of rapid onset. The blood pressure of one of these cases did not fall after operation; in the other it fell to normal. In other cases the vascular lesions have also been reversible and the blood pressure has reverted to normal after removal of the tumour. In two cases,[89, 337] after falling to, or close to, normal readings following operation, the blood pressure was again raised when the patients were examined 4 months later. Nephrosclerosis was reported in the renal biopsy from one of these cases,[89] it was also present in Conn's original case in whom the blood pressure returned to, and remained, normal after operation. In two of the three patients studied by Dustan, Corcoran and Page,[118] the blood pressure did not fall after removal of the tumour.

Tissue biopsies of patients with this syndrome show increased intracellular sodium and decreased intracellular potassium (see below). The *hypertension present in this syndrome may be related to this electrolyte* shift in arterioles, making them more sensitive to the pressor effect of noradrenaline, an effect observed following the administration of deoxycorticosterone.[274] The postulate that the increased intracellular sodium concentration is due to the action of aldosterone is in conflict with the experimental evidence provided by Woodbury and Koch,[544] who found that aldosterone lowered rather than raised the intracellular sodium concentration in rat muscle.

The majority of cases experienced polyuria, polydipsia and nocturia and exhibited hyposthenuria with an inability to concentrate the urine when deprived of water, they likewise did not respond to the administration of vasopressin by an increase in the osmolarity of the urine. This type of vasopressin-resistant polyuria has been described in the nephropathy of potassium depletion [386, 426] A similar syndrome develops in dogs treated for long periods with deoxycorticosterone,[344] in experimental animals on a potassium-deficient diet[154, 456] or in patients with potassium depletion due to intractable diarrhoea.[368] Anti-diuretic hormone is present in normal amounts in patients with Conn's syndrome.[493]

Proteinuria was present in the majority of cases This also occurs in the nephropathy of potassium depletion.

The polydipsia has been variously ascribed to the result of thirst caused by intracellular dehydration[illegible] or to hypernatraemia.[illegible] The injection of deoxycorticosterone was shown by Muntwyler, Mautz and Griffin[illegible] to result in a decrease of intracellular water resulting from loss of intracellular potassium without compensatory gain of sodium.

Tetany has been reported in six cases.[illegible] In three of these, the serum calcium concentration was normal and the serum phosphorus concentration was in the lower part of the normal range. In the other cases, values for these determinations are not given. All these cases were alkalotic. In another case,[illegible] where alkalosis was not present tetany could be produced by loading the patient with potassium citrate. The presence of tetany in the face of normal serum calcium and phosphorus levels may be explicable on the basis of an extracellular alkalosis with intracellular acidosis. In one case,[illegible] the tetany was relieved by rebreathing into a paper bag.

Aldosterone Excretion in Conn's Syndrome

Elevated values for aldosterone excretion, estimated both by bioassay and physicochemical methods, have been reported in some cases of this syndrome, as would be expected from the designation given to it by Conn. Normal values *for aldosterone excretion* have, however, been found even in cases in which the disease is apparently active[illegible] and in which adrenalectomy has been curative. Potassium depletion depresses the secretion of aldosterone. Attempts have been made to explain the normal excretion of aldosterone in cases of Conn's syndrome on this basis.

It is surprising to find that the urinary output of aldosterone may be much less in 'primary aldosteronism' than it is in 'secondary aldosteronism'. [illegible] hr.[117] In [illegible] retaining [illegible] method[illegible] and a daily excretion of 300-2300 μg deoxycorticosterone equivalents was found in five consecutive urine samples, the range found in normal subjects by this method was 24 to 115 μg. deoxycorticosterone equivalents per 24 hours. Bioassay of the aldosterone-like activity of the tumour gave a value of 8 7 μg /g.; this figures is based on the assumption that aldosterone has thirty times the activity of deoxycorticosterone acetate. Conn[illegible] states that paper chromatographic study of extracts of the tumour showed that the active material was aldosterone.

Fluctuations in the daily excretion of aldosterone were noted in the case of patient M. L, discussed below (p. 95), who had an adrenocortical adenoma. Fluctuations in the secretion of corticosteroids also occur in

Cushing's syndrome. It is therefore possible that fluctuations in the daily excretion of aldosterone occur in Conn's syndrome, and that the cases in which a normal amount of aldosterone has been found in the urine have had periods in which aldosterone excretion has been grossly excessive.

Nevertheless, the finding of a normal excretion of aldosterone in the urine of these cases is disturbing and raises the question of the significance of aldosterone in the aetiology of this syndrome. There is the possibility that the disorder should be ascribed to an adrenocortical hormone not yet described, or to an excess of corticosterone or other known steroid, the physiological properties of which in man have not been well defined. It is more probable that the syndrome is the result of the combined effect of aldosterone and another steroid such as corticosterone. This may have been the case in the patient described by Mader and Iseri[illegible] since analysis of the tumour showed a large quantity of corticosterone (33 µg./g.). The simultaneous administration of hydrocortisone and aldosterone was found by Ross and colleagues[illegible] to result in a much greater excretion of potassium than when either of these compounds was given alone. This, indeed, is also true of the simultaneous infusion of corticosterone and aldosterone over an 8-hour period.[illegible] The long-term administration of these two steroids may of course give a different result.

Whatever the nature of the defect in Conn's syndrome, it is certain that it is the result of a disorder of secretion of the adrenal cortex, since adrenalectomy is curative, but that it is in fact due solely to hyperaldosteronism is at present far from proven. For this reason it is preferable to refer to this disorder as 'Conn's syndrome' until the nature of the abnormality becomes clarified. Luetscher,[illegible] for this reason, prefers the term 'syndrome of mineralocorticoid excess'.

Sodium Metabolism in Conn's Syndrome

Despite the high levels of aldosterone excreted in the urine of some cases, sodium excretion in the urine is not correspondingly reduced and oedema is not present. It is apparent that the renal tubule in Conn's syndrome is refractory to the sodium-retaining activity of aldosterone. Thus the balance data in many cases show that urinary sodium excretion may range from 100 to 200 mEq a day. A similar refractoriness is not seen in the effects of aldosterone on the secretion of sodium and potassium by the epithelium of salivary and sweat glands or of the intestinal tract, since low sodium/potassium ratios in saliva and stool are features of this syndrome and have been used as criteria of diagnostic importance.

In one case[illegible] removal of the tumour was followed by a phase of hypoaldosteronism, with inability to conserve sodium; this was ultimately

corrected by placing the patient on a low sodium intake which provided a stimulus to aldosterone secretion.

The rate of disappearance of radioactive sodium ([illegible]Na) from plasma was found by Crane, Short and Peterson[illegible] to be greatly prolonged in a patient with Conn's syndrome. The biological decay rate was 40 days when measured before operation; after removal of the adenoma it fell to 10 days (Normal = 8 to 14 days).

Details of the renal tubular response to reduction of sodium intake in cases of Conn's syndrome do not appear to be available.

Potassium Metabolism in Conn's Syndrome

Despite the very potent sodium-retaining properties of aldosterone to which most attention has been paid in assessing its physiological effects, the most prominent manifestations of Conn's syndrome are those of potassium wasting by the kidney, with resultant hypokalaemia leading to muscular weakness and nephropathy. If it is assumed that the syndrome is in fact due to the excessive production of aldosterone, it is still not clear from the clinical studies performed to date with aldosterone why potassium depletion predominates over sodium retention.

The administration of aldosterone to normal subjects and to subjects with Addison's disease for short periods (4 to 6 days) results in sodium retention and weight gain, with very little change in potassium excretion.[illegible] This is similar to the effects of the short-term administration of deoxycorticosterone.[illegible] On the other hand, the prolonged administration of large doses of deoxycorticosterone to normal dogs[illegible] and to patients with Addison's disease[illegible], [illegible] results in a syndrome characterized by vasopressin-resistant polyuria, hypertension and periodic muscular paresis or paralysis due to potassium deficiency; in other words, in a syndrome closely resembling that described by Conn. August and colleagues[illegible] have shown that the long-term effects of the administration of aldosterone to normal subjects over a period of 2 and 4 weeks resemble to some extent those of deoxycorticosterone in causing initial sodium retention and potassium diuresis, compared with the control period, followed by a return to control levels of excretion. Cessation of aldosterone administration was followed by sodium diuresis and potassium retention In one subject the combined loss of potassium in stool and urine did not exceed the potassium intake during the period of aldosterone administration. In the other subject there was a negative potassium balance of 9.7 mEq /day. On these figures it would take a very long time to produce a potassium-deficit of the degree seen in patients with Conn's syndrome. At the present time, in the absence of evidence to the contrary, it must be assumed that the potassium

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Despite the high levels of aldosterone excreted in the urine of some cases, sodium excretion in the urine is not correspondingly reduced and oedema is not present. It is apparent that the renal tubule in Conn's syndrome is refractory to the sodium-retaining activity of aldosterone. Thus the balance data in many cases show that urinary sodium excretion *may range from 100 to 200 mEq. a day. A similar refractoriness is not seen* [illegible]

In one case[444] removal of the tumour was followed by a phase of *hypoaldosteronism, with inability to conserve sodium; this was ultimately*

by the method of isotope dilution are reported in two cases of Conn's syndrome. The results, given below, show that there was an increase of total exchangeable sodium and decrease of total exchangeable potassium in these cases. Removal of the tumour restored electrolyte composition to normal.

Reference	*State*	Na_e *(mEq)*	K_e *(mEq)*	*Na_e/K_e
Chalmers *et al.*[59]	Pre-operative, after depletion of potassium	2409	1441	1.7
Chalmers *et al.*[59]	Pre-operative, after repletion of potassium	2150	1785	1.2
Chalmers *et al*[59]	Post-operative	1893	2088	0.9
Crane *et al*[92]	Pre-operative	3041	2084	1.5
Crane *et al*[92]	7 months post-operative	2691	2337	1.2
Crane *et al*[92]	1 year post-operative	2623	2454	1.1

* Normal values for the ratio Na_e/K_e range from 0.8 to 1.1.

Tissue Analyses in Conn's Syndrome

Muscle biopsies were performed in five patients with Conn's syndrome; the results of tissue analyses in these cases are shown below.

Reference	*State*	*Tissue analysis*	*Normal range*
Buchem[491]	Pre-operative	Na = 52.4 mEq/kg. wet wt	26–30
		K = 67.5 mEq/kg wet wt.	91–107
Conn[67]	Pre-operative	Na = 43–47 mEq/kg wet wt	as above
		K = 58–66 mEq/kg wet wt	as above
Conn[67]	Post-operative	Na = 32 mEq/kg wet wt.	as above
		K = 76 mEq/kg wet wt.	as above
Chalmers *et al*[59]	Pre-operative	Na = 215 mEq/kg fat free dry tissue	114–119
		K = 386 mEq/kg fat free dry tissue	435–481
Milne *et al.*[327]	Pre-operative	Na = 220 mEq/kg fat free dry solid	as above
	Case 1	K = 390 mEq/kg fat free dry solid	as above
Milne *et al*[327]	Pre-operative	Na = 155 mEq/kg fat free dry solid	as above
	Case 2	K = 405 mEq/kg fat free dry solid	as above

It will be seen that in all cases there is an increased content of sodium, and a decreased content of potassium within the muscle cell.

Tissue analysis in adrenalectomized dogs with the 'diabetes-insipidus-like syndrome' produced by the prolonged administration of deoxycorticosterone likewise has shown that intracellular potassium was replaced in part by sodium.

Effect of Corticotrophin Administration on Excretion of Sodium and Potassium

Conn[67] noted in his case that the administration of corticotrophin resulted in considerable sodium retention, and slight potassium diuresis,

depletion of this syndrome is due to the excessive secretion of aldosterone. However, in the case of patient M. L. discussed below (p. 95), who was certainly suffering from long-term overproduction of aldosterone, the only manifestations of the effects of this hormone was sodium retention and not potassium depletion. Cases of 'secondary hyperaldosteronism', whose excretion of aldosterone may be higher than that of cases of 'primary hyperaldosteronism', likewise do not suffer from potassium depletion. It may be that the dietary restriction of sodium to which these patients are subjected protects them from the potassium-losing activity of the hormone as does sodium depletion from the potassium-depleting effect of prolonged deoxycorticosterone administration.[421]

Hypokalaemia has been present at some period in all reported cases of Conn's syndrome, serum-potassium concentrations as low as 1.4 mEq./l. being recorded in one instance.[112] The serum potassium concentration is said to be lower than would be expected from the degree of potassium depletion, judged by the serum level found in cases of potassium depletion due to other causes.[117] Conn[97] noted, as have Mader and Iseri[311] and Crane, Short and Peterson[91] that it is difficult in this syndrome to bring the serum potassium levels back to normal by the administration of potassium chloride; others[113] have been able to restore the serum level rapidly to normal by potassium loading.

The low serum potassium level is the result of an excessive loss of potassium by the kidneys, and to a lesser extent by the bowel. Despite the low serum potassium levels, these patients fail to conserve this electrolyte. They similarly fail to conserve potassium when placed on a low potassium intake. In Evans and Milne's case[133] the total potassium deficit was over 1000 mEq. Balance data showed that 70% of the potassium lost from the intracellular compartment had been replaced by sodium; the remaining cation deficit was made good by the movement of hydrogen ion into cells, accounting for the intracellular acidosis and extracellular alkalosis present in this syndrome. This electrolyte shift is of the same order as that noted by Darrow and associates[97] in potassium deficiency with alkalosis and also subsequently by Cooke and co-workers[81] in potassium deficiency in rats produced by the ingestion of deoxycorticosterone while on a potassium-deficient diet. The magnitude of the potassium depletion can be assessed by placing the patient on a known potassium intake, 250 to 300 mEq./day, and measuring the cumulative balance until potassium excretion balances the intake.

TOTAL EXCHANGEABLE SODIUM AND POTASSIUM IN CONN'S SYNDROME

Measurements of total exchangeable sodium (Na_e) and potassium (K_e)

their patient had a considerable exchange of intracellular potassium for sodium, yet presented no extracellular alkalosis.

Reaction of the Urine in Conn's Syndrome

The reaction of the urine in Conn's syndrome is neutral or alkaline, and remains so during concentration tests, when the urine is normally acid. Diurnal variation of urinary pH is absent.[114] In hypokalaemia due to other causes the urine is acid, despite the extracellular alkalosis. The intracellular concentration of potassium has been considered to be the determinant of urinary pH,[18] a low intracellular potassium concentration in the tubule cell resulting in increased reabsorption of potassium in exchange for hydrogen ion. The alkalinity of the urine in Conn's syndrome is in disagreement with this view. Dustan, Corcoran and Page[116] dispose of this difficulty by postulating that aldosterone increases the ionization of potassium within the cell, making the assumption that potassium exists within the cell in a bound nonionized form.

The ability of the kidney to acidify the urine is retained in Conn's syndrome, although this function may be somewhat defective.[68, 118] Urinary pH has been observed to fall to 5.1 during both sulphate[118] and ammonium chloride[68] loading.

The ability to form ammonia is preserved and indeed is considerably increased in Conn's syndrome,[117] the ammonia content of the urine being greater for a given pH than occurs in normal subjects.[118a]

Correction of the alkalosis of Conn's syndrome by the administration of potassium chloride, or by the removal of the adrenocortical adenoma, results in the liberation of hydrogen and sodium ions from cells, with replacement by potassium ions. This results in the excretion of a highly acid urine. Ammonia production by the kidney is now decreased as potassium is repleted.

Renal Function in Conn's Syndrome

The glomerular filtration rate, measured by either the clearance of inulin (C_{In}) or of creatinine (C_{Cr}) is usually reduced in Conn's syndrome, as is the renal plasma flow as measured by the clearance of para-aminohippuric acid (C_{PAH}). The tubular extraction of p-aminohippurate may be defective in potassium depletion so that the clearance of this substance may not be a true reflection of renal plasma flow.[117]

Following removal of the tumour these parameters of renal function are further depressed, but improvement is usually seen 4 to 6 months after operation.

Potassium depletion in Conn's syndrome is due to a renal leakage of

lasting for 3 days. A sodium diuresis then occurred, although corticotrophin administration continued. A slight potassium diuresis accompanied the sodium diuresis. The administration of cortisol had a similar but less dramatic effect. Conn suggests that this indicates that cortisol in high dosage antagonizes the effect of aldosterone on the renal tubule. Crane, Short and Peterson[illegible] have also noted sodium retention followed by sodium diuresis during the administration of corticotrophin. Eales and Lindner[illegible] observed sodium retention followed by diuresis during the administration of prednisone to their case; no sodium diuresis was observed by these authors during the administration of corticotrophin over a 4-day period. Skanse and colleagues[illegible] observed increased sodium excretion, but no change in potassium excretion, during corticotrophin administration. Hellem[illegible] appears to be alone in observing retention of potassium during the administration of both cortisone and corticotrophin.

Magnesium Metabolism in Conn's Syndrome

The serum magnesium concentration was low in some cases of Conn's syndrome in which this measurement has been made. Mader and Iseri[illegible] report values of 1.12 mEq./l. (normal range 1.3–1.9 mEq./l.). Evans and Milne[illegible] state that the serum magnesium concentration in their case was 'below normal'. Hewlitt and co-workers[illegible] on the other hand, report values of 2.5 mEq./l. in one case, and 2.2 mEq./l. in another.

A 21-day magnesium balance performed by Mader and Iseri[illegible] on an intake of 12–14 mEq./day showed a persistently negative balance due to excessive loss in the stool. Renal loss of magnesium was also higher than normal.

Alteration in the urinary excretion of magnesium could not be demonstrated in secondary hyperaldosteronism induced by placing normal subjects on a low salt diet.[illegible] The authors conclude that the magnesium deficiency noted in patients with Conn's syndrome may be connected with the associated potassium depletion.

Extracellular Alkalosis in Conn's Syndrome

A hypochloraemic alkalosis is not an essential feature of Conn's syndrome, being absent in the cases presented by Chalmers and colleagues[illegible] and Crane and colleagues.[illegible] Many cases, however, do exhibit a raised arterial pH, raised alveolar carbon-dioxide content and raised plasma carbon-dioxide combining power. The extracellular alkalosis has been ascribed to the presence of an intracellular acidosis as described above, but muscle biopsies performed by Chalmers and colleagues[illegible] showed that

of 300-400 mg./100 ml; the second had a fasting blood-sugar concentration of 107 mg/100 ml. with an excessive rise and delayed fall of blood-sugar level following an oral glucose load; the third case had a fasting blood-sugar concentration of 70 mg/100 ml. with a normal rise but delayed fall of glucose levels following an oral glucose load. In the second and third of the above patients the glucose-tolerance curve returned to normal after removal of the adrenocortical adenoma. In the first case the glucose-tolerance test was still abnormal 3 months after operation.

A normal rise of blood-sugar concentration with a delayed fall following a 50 g. glucose load was also observed in the case reported by van Buchem, Doorenbos and Elings.[401] A post-operative glucose-tolerance test is not reported. A fasting blood-glucose concentration within the upper range of normal was also noted in the cases reported by Crane, Vogel and Richland[92] and by Crane, Short and Peterson.[92]

Severe diabetes mellitus in a presumed case of Conn's syndrome has been reported by Sorce and Whitstone.[417a]

The abnormality of glucose tolerance seen in these cases may be related to potassium deficiency. Potassium-depleted rats show a decreased glucose tolerance.[46] As discussed above (p. 55) aldosterone has a stimulating effect on gluconeogenesis, but its activity in this respect is only about one-third of that of hydrocortisone, weight for weight. The small quantities of aldosterone secreted daily would not be expected to have any effect on glucose metabolism. It is, of course, possible that excessive secretion of another steroid may be responsible for the abnormal glucose tolerance seen in the patients discussed above.

Pathology of Tumour

Descriptions of the cellular morphology of the tumour removed from cases of Conn's syndrome state that in some cases the cells resembled those of the zona glomerulosa[210] whilst in others it resembled the zona fasciculata;[66, 212] other authors report that the tumour contained tissue resembling both these layers. Conn[73] states that there was atrophy of the zona fasciculata in the remainder of the gland. Hewlitt and co-workers,[211] on the other hand, found no atrophy of the zona fasciculata. Fine and co-workers[149] state that there was no atrophy of any zone in the remainder of the gland. Milne and co-workers[317] report atrophy of the remaining gland, especially of the zona glomerulosa.

Cases of Conn's syndrome have been described in which no tumour was present. It is of interest that these have all been children or adolescents. The single adrenal gland removed from a 13-year-old girl by Holten and

potassium which persists in the presence of hypokalaemia. This tubular defect is presumably due to the presence of excessive amounts of, or hypersensitivity to normal amounts of, aldosterone or other adrenal steroid.

Potassium wasting implies either a defective reabsorption of filtered potassium or an excessive tubular secretion of this ion. The second mechanism is perhaps more likely to be at fault since, in a study of two patients with Conn's syndrome, Relman and Schwartz[illegible] noted that the renal excretion of potassium was only excessive when the sodium intake was high. On a normal intake of potassium and a low intake of sodium the potassium balance became positive, suggesting that the excreted potassium was derived largely from ion exchange with sodium. Some doubt is thrown on this simple explanation, however, by the demonstration that an effect on sodium excretion can be dissociated from an effect on potassium excretion when aldosterone is infused into the renal artery.[illegible]

The renal tubule in Conn's syndrome also shows an inability to respond to the sodium-retaining activity of aldosterone. High doses of deoxycorticosterone likewise fail to cause oedema when administered to cases of Conn's syndrome, according to van Buchem, Doorenbos and Elings.[illegible]

Amino-aciduria has not been observed in this syndrome.

Renal Pathology

The renal tubules in Conn's syndrome microscopically show dilatation and vascular degeneration. Nephron dissection by the method of Darmady[illegible] in one case[illegible] showed localized vacuolation and distortion limited to the proximal tubule. Similar anatomical changes in both the proximal[illegible] and distal[illegible] tubules have been noted in chronic potassium deficiency. Clinical and histological evidence of pyelonephritis was present in the cases described by Milne,[illegible] by Eales[illegible] and by Kretschmer[illegible] and has been described in potassium depletion due to other causes.[illegible] The renal lesions are reversible and a return to normal is possible when the patient is repleted with potassium, provided concomitant pyelonephritis has not resulted in irreversible fibrotic changes.

Carbohydrate Metabolism in Conn's Syndrome

Reduction of carbohydrate tolerance has been reported in Conn's syndrome. In the majority of cases a glucose-tolerance test has not been performed. McCullagh[illegible] reports observations on three patients with Conn's syndrome, one of whom had a fasting blood-sugar concentration

of 300-400 mg/100 ml; the second had a fasting blood-sugar concentration of 107 mg/100 ml with an excessive rise and delayed fall of blood-sugar level following an oral glucose load; the third case had a fasting blood-sugar concentration of [illegible] mg/100 ml with a normal rise but delayed fall of glucose levels following an oral glucose load. In the second and third of the above patients the glucose-tolerance curve returned to normal after removal of the adrenocortical adenoma. In the first case the glucose-tolerance test was still abnormal 3 months after operation.

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DISCUSSION OF CONN'S SYNDROME AND OTHER HYPERTENSIVE-HYPOKALAEMIC SYNDROMES

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The situation which Conn appeared to clarify has, however, now been confused once again by the recognition of the presence of hypokalaemia, and its sequelae, in many cases of hypertension in whom no primary adrenal abnormality, that is, an adenoma, can be demonstrated.[illegible] Hilden and Krogsgaard[illegible] make the interesting observation that in some of these cases reduction of the blood pressure by means of hypotensive drugs results in a reversion of the serum potassium concentration to levels within the normal range, without the administration of potassium supplements. An increased secretion of aldosterone has been reported in three cases in whom adrenalectomy restored the plasma potassium concentration to normal, but had no effect on the blood pressure.[illegible] Five [illegible]

urine and had a secretion rate, measured by dilution of tritiated aldosterone, of only 4 μg./24 hr. In this patient, in whom no renal abnormality could be demonstrated, bilateral adrenalectomy was without effect on either blood pressure or plasma electrolyte abnormalities, neither did it change the pattern of renal excretion of corticosteroids. It is believed that an adrenocortical tumour has still to be located. In all these cases there was a grossly abnormal pattern of corticosteroid excretion, but no sodium-retaining steroid has so far been isolated from the urine. One of these patients had a phaeochromocytoma which was causing unilateral renal ischaemia by pressure of the renal artery. Removal of the tumour and of the affected kidney resulted in a fall of blood pressure to normal and in a return of the plasma potassium concentration to normal without the addition of potassium supplements. The functional abnormalities of the adrenal cortex and the hypokalaemia of the cases discussed in this paragraph would appear to be secondary to the hypertension.

Peterson[310] was described as histologically normal as were those removed from a 13-year-old boy by Bartter and Biglieri.[26] The glands removed from a 17-year-old boy by van Buchem, Doorenbos and Elings[402] showed hyperplasia of the zona fasciculata, as did those of a 9-year-old boy described by Kretschmer, Dickinson and Karl.[243]

Hormonal Analysis of Tumour

Analysis of the aldosterone content of the tumour in Conn's original case[88] showed that it contained 8.7 µg. of aldosterone per gramme of tumour tissue. The tumour of Mader and Iseri's case[271] contained 1.4 µg./g., that of Eales[133] 5.6 µg./g. The aldosterone content of the hyperplastic glands removed by van Buchem[402] contained 0 25 µg /g.

Neher[288] reports the results of the analysis of three adenomas from cases of Conn's syndrome. The aldosterone content of these tumours was 1.4, 1.05 and 1.08 µg per g. The content of cortisol was 9.0, 3.5 and 4.0 µg /g. and of corticosterone 32 5, 1.4 and 2 0 µg./g. Neher[288] gives the following figures for the steroid content of a normal adrenal gland: aldosterone, 0.05 µg /g ; cortisol, 3.9 µg./g. and corticosterone, 2.9 µg./g.

Taking into account a probable daily secretion of 200 µg ,[18] it would appear from these figures that the adrenal cortex does not store aldosterone to any extent

Miscellaneous Obervations in Conn's Syndrome

The urinary excretion of 17-hydroxycorticoids, 17-ketogenic steroids and 17-ketosteroids is normal in Conn's syndrome. Plasma corticotrophin concentration is also said to be normal [402]

Retardation of growth has been observed in children with this syndrome [310, 402] This may be the result of prolonged potassium deficiency. It occurs in rats fed diets deficient in potassium.

A lowered arterial oxygen saturation (87%) was noted by Foye and Feichmeier [156] A normal arterial oxygen saturation was found by van Buchem and co-workers.[402]

Changes in the gastric juice were noted by van Buchem and co-workers.[402] Pre-operatively their patient had a histamine-fast achlorhydria, whereas post-operatively he had a very high secretion of hydrochloric acid.

A greatly increased rate of excretion of uropepsin was noted by Crane, Short and Peterson,[82] falling to normal post-operatively. Crane, Vogel and Richland[83] similarly found a high urinary uropepsin excretion in a subsequent case.

Discussion of Conn's Syndrome and other Hypertensive-Hypokalaemic Syndromes

[illegible]

accepted as a disease entity in its own right. Conn clearly differentiated this syndrome from cases, previously described,[illegible] of potassium-losing nephritis, characterized by excessive renal loss of potassium in the presence of potassium depletion and hypokalaemia, in which it was believed that the primary lesion resided in the kidney. Some of these alleged cases of 'potassium-losing nephritis' have undoubtedly been examples of Conn's syndrome, as was that reported by Evans and Milne.[illegible]

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The existence of cases of hypertension with hypokalaemia, such as those described above, adds to the already difficult task of diagnosing cases of Conn's syndrome. Into which category does one place the case described by Hilton and colleagues,[217b] for example? The syndrome described by Conn would seem to be a true disease entity, since the removal of an adrenocortical tumour has been curative of both hypertension and electrolyte disturbance in many cases. It is possible that long continued hypertension can be the stimulus to adenoma formation in the adrenal cortex, as was probably the case with long continued oedema,[200] but if this were the cause of Conn's syndrome there is no reason to expect that removal of the adenoma would result in a return of blood pressure to normal. It may, however, be of significance in this connection to note that no case of Conn's syndrome due to an adenoma has yet been reported in a child.

The distinction between the two syndromes of hypertension associated with hypokalaemia is difficult to make. Exclusion of other causes of hypertension by means of intravenous pyelography, aortography and estimation of catechol amines is essential. The administration of amphenone B, leading to correction of the plasma potassium and bicarbonate abnormalities, suggested as helpful in the diagnosis of Conn's syndrome,[210] does not distinguish between the two conditions. Peri-renal oxygen insufflation and tomography may demonstrate the presence of an adrenal tumour. At present the only certain method of diagnosing Conn's syndrome is by laparotomy and inspection of both adrenal glands.

Prognosis

This syndrome would appear to be benign unless malignant hypertension supervenes. Milne's first case had a history of muscular weakness for 12 years before removal of the tumour.[102] The development of malignant hypertension is a definite hazard, and in many cases the hypertension, temporarily relieved by removal of the tumour, returned within a few months of operation In one case, with hypertension for 25 years, removal of the adenoma was followed by a fall of blood pressure to normal.[217 (case 1)]

Following removal of the tumour potassium retention and sodium diuresis occur, these changes were noted within a day in Conn's patient.[47] The serum potassium concentration had become normal by the sixth day.

The patient reported by Crane, Short and Peterson[92] experienced a phase of hypotension following operation which necessitated the administration of vasoconstrictor substances The administration of potassium

chloride to this patient was followed by rapid improvement of the circulatory collapse; the authors suggesting that the injected potassium passed into cells to release sodium which was then available for expansion of the extracellular space. This case illustrates the importance of giving supplements of potassium chloride during the immediate post-operative period.

SECONDARY HYPERALDOSTERONISM

CONN,[11] who was responsible for the original classification of hyperaldosteronism into 'primary' and 'secondary', employed the term 'primary aldosteronism' to denote as excessive secretion (excretion) of aldosterone due to an abnormality of the adrenal cortex, usually the presence of an adenoma. The term 'secondary aldosteronism' denoted the secretion (excretion) of excessive amounts of aldosterone due to a stimulus which resided outside the adrenal cortex, acting through the physiological mechanisms which control aldosterone secretion. Some conditions under which 'secondary aldosteronism' occurs are listed in the Table below.

SECONDARY ALDOSTERONISM

1. Associated with oedematous states
   - (a) Congestive heart failure
   - (b) The nephrotic syndrome
   - (c) Cirrhosis
   - (d) 'Idiopathic' oedema

2. Induced by excessive fluid loss
   - (a) Untreated diabetes insipidus
   - (b) Untreated diabetes mellitus
   - (c) 'Salt-losing nephritis'
   - (d) Dietary sodium restriction
   - (e) Diuretics
   - (f) Phlebotomy
   - (g) Excessive exercise

Details of aldosterone excretion occurring in most of these conditions have already been discussed (Chapter III). Possible reasons for the increased excretion of aldosterone in these circumstances are discussed in Chapter XI. The significance of aldosterone in some interesting cases of 'idiopathic oedema' and in psychiatric disturbances is discussed below.

'IDIOPATHIC' OEDEMA WITH HYPERALDOSTERONISM

In addition to the well-recognized occurrence of hyperaldosteronism in oedematous states associated with cardiac, renal or hepatic failure, interest has been directed to the excretion of aldosterone in a group of patients suffering from oedema, the underlying cause of which is not known, no cardiac, renal or hepatic disease being detectable. The syndrome presented by these patients is that of generalized oedema, with

normal plasma proteins, normal serum electrolyte concentrations, normal blood pressure and a very low concentration of sodium in the urine.

Such a case was published by Mach, Fabre, Muller, Neher and Borth.[illegible] The patient was a woman aged 46 who had a 26-year history of generalized oedema accompanied by headache, coldness of the extremities, attacks of amblyopia and transitory aphonia. When placed on a sodium balance it was found that she retained sodium and gained weight whenever her sodium intake exceeded 70 mEq. In this patient a moderate rise of blood pressure was noted during periods of sodium retention. The amount of aldosterone excreted in the urine measured by the method of Neher and Wettstein[illegible] on urine extracted immediately after acidification, was ten times the upper limit of normal in two estimations when the patient was on a sodium intake of about 85 mEq., and three times the upper limit of normal when the sodium intake was 170–200 mEq of sodium a day.

Mach[illegible] later reported a second case. She was a 37-year-old woman in whom generalized oedema developed a few weeks after an emotional shock. This patient had normal serum sodium and potassium concentrations with a carbon-dioxide combining power of 32 mEq./l. Her plasma albumin concentration was only 2.3 g./100 ml.; this is reported to have later increased. Her urinary excretion of aldosterone was 109 µg./24 hr.; the sodium intake at the time the urine specimen was collected is not stated. This patient failed to have a worthwhile sodium diuresis when loaded with 250 mEq. of sodium, only 35 mEq. being excreted. Her daily excretion of aldosterone fell during sodium loading from 109 µg. to 20 µg. when the sodium load was given. Increasing fluid intake in this patient when on a sodium [illegible]

to [illegible]

[illegible]

rone-secreting adrenocortical adenoma has been presented by Ross, Crabbé, Renold, Emerson and Thorn.[illegible] The patient (M. L.) was a 38-year-old woman who had a 10-year history of generalized oedema, ascites and bilateral pleural effusions. She had no demonstrable cardiac, renal or hepatic disease. Her serum electrolytes were normal, as was her blood pressure. Her plasma albumin concentration on one admission was 3.1 g./100 ml. when the albumin content of her pleural fluid was 2.1 g./100 ml. On other admissions the plasma albumin was within the normal range. The aldosterone excretion in the urine of this patient was very high, ranging from 20 to 120 µg /24 hr. even on a sodium intake of 135 mEq. a day. Her urinary sodium excretion was less than 5 mEq /day and she had a very poor sodium response to diuretics. The administration of

amphenone resulted in a brisk sodium diuresis on three occasions, suggesting a causal relationship between her hyperaldosteronuria and her sodium retention. Adrenal exploration was therefore performed and an adrenocortical adenoma was found and removed. Following this operation aldosterone disappeared from her urine and she had a sodium diuresis and lost 14 kg. in weight. Six weeks after removal of the adenoma, aldosterone was again found in the urine in amounts of up to 30 µg./24 hr. She could now, however, adjust her sodium excretion to balance the sodium intake. Six months after operation, her urinary output of sodium had fallen to less than 1 mEq./day and her output of aldosterone ranged from 15 to 65 µg./24 hr. The administration of amphenone again resulted in a sodium diuresis. Removal of her remaining adrenal gland again resulted in a brisk sodium diuresis and weight loss of 16 kg. in a month. Further weight loss resulted in symptoms referable to hyponatraemia. She was therefore stabilized on a sodium intake of 205 mEq./day, hydrocortisone and fluorohydrocortisone. Some oedema still persists.

The formation of oedema in this patient appeared to result from an abnormality of capillary permeability with respect to protein, a defect well substantiated by the studies of Emerson and Armstrong.[194] This defect appears to have initiated an excessive secretion of aldosterone, perhaps as a result of a low effective blood volume, causing extreme sodium retention which perpetuated her oedema. The long-continued stimulus to aldosterone secretion eventually gave rise to the formation of a functioning adenoma on one side. In this respect this case could be labelled 'primary secondary aldosteronism'.

It is of interest to note that aldosterone secretion by the contralateral adrenal was suppressed by the presence of the adenoma, although the stimulus to secretion was acting on both adrenal cortices. When the adenoma was removed, the remaining adrenal cortex commenced to secrete excessive quantities of aldosterone.

Further cases of unexplained oedema in emotionally labile women, associated with hyperaldosteronism, have been reported by Luetscher and Lieberman.[294] The oedema in these women fluctuated with emotional stress and fatigue. These patients had normal plasma electrolyte and albumin concentrations; four of the five had normal blood pressures. All had increasing quantities of aldosterone in the urine and did not respond to sodium loading by more than a slight reduction of aldosterone excretion; their 'cut-off' mechanism appears to be set at a higher level of sodium intake than normal.

In the discussion of this paper, Altschule[4] reported that patients with catatonic schizophrenia, with normal cardiac and renal function, become

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isolated hypoaldosteronism, is reported by Skanse and Hökfelt.[444] The patient was a housewife, aged 56, who had an 11-year history of frequent attacks of severe dizziness, weakness and easy fatiguability. She was not pigmented. Her blood pressure was 90/60 mm. Hg., but there was no postural hypotension. The only biochemical abnormality was a serum sodium concentration of 136 mEq /l. (normal range for their laboratory 141-150 mEq./l.); the serum potassium concentration was 3.5 mEq /l. Urinary excretion of 17-ketosteroids and 17-ketogenic steroids was normal and there was a brisk response to the administration of corticotrophin.

The quantity of aldosterone excreted in the urine was too low to be measurable by the physicochemical method of Neher and Wettstein.[344] There was no rise of aldosterone excretion when the patient was placed on a sodium intake of less than 10 mEq. daily. Despite this, the amount of sodium excreted in the urine decreased to less than 10 mEq /24 hr. on the sixth day of the restricted sodium intake. There was a considerably increased excretion of free hydrocortisone and cortisone during the period of sodium restriction; this does not normally occur.[94] There was no fall of serum sodium concentration during the period of low sodium intake. The patient responded to the administration of deoxycorticosterone acetate, 3 mg./day by intramuscular injection, by a rise of blood pressure and a slow increase of serum sodium concentration to 144 mEq /l. Her episodes of dizziness continued, but she had no fainting attacks whilst receiving deoxycorticosterone acetate.

The clinical picture presented by this patient is the opposite of that seen in Conn's syndrome, with the exception that the serum potassium concentration was at the lower limit of the normal range. Compensation for the absence of the secretion of aldosterone in this patient may have been afforded by an excessive secretion of hydrocortisone. The ability of the kidney to conserve sodium in the absence of a measurable excretion of aldosterone is noteworthy and supports the contention of Crabbé, Ross and Thorn[91] that factors other than aldosterone play an important role in the renal tubular handling of sodium during a period of restricted dietary intake of sodium.

An 11-year-old boy suffering from hypertension, hypokalaemia, hypernatraemia and alkalosis has been studied by the author.[404] Aldosterone was absent from the urine and his aldosterone secretion rate, measured by dilution of tritiated aldosterone, was likewise barely measurable. His urine showed an abnormal pattern of steroid excretion. Despite the absence of aldosterone, this patient was able to adapt normally a low sodium intake, resembling the patient of Skanse and Hökfelt.[444] Adapta-

HYPOALDOSTERONISM

A CASE report which suggests the existence of an *isolated* deficiency of adrenocortical production of aldosterone, with normal secretion of other adrenocortical hormones, has recently been published by Hudson, Chobanian and Relman[333] under the title 'Hypoaldosteronism'.

This 71-year-old man with atrioventricular block suffered from attacks of cardiac arrest which coincided with increases of serum potassium concentration to the order of 6 to 7 mEq./l., a much lower level of hyperkalaemia than usually results in cardiac standstill. These attacks were precipitated by placing the patient on a low salt diet for treatment of congestive failure. This patient's difficulty in controlling the level of his serum potassium appeared to be due to an inability to secrete aldosterone in adequate quantity. On a normal intake of sodium and potassium the excretion of aldosterone, measured by the method of Hernando and co-workers,[334] was less than 0.5 µg./24 hr. When placed on a low sodium diet, the values were less than 0.5, 3.3 and 2.5 µg./24 hr. When given corticotrophin whilst on a low sodium diet the excretion of aldosterone was 4.8 µg./24 hr. His urinary excretion of 17-hydroxycorticoids was 5.9 to 8.2 mg./24 hr., and of 17-ketosteroids 6.4 to 6.9 mg./24 hr., values which are within normal limits; he had a normal response of these hormones to the administration of corticotrophin. Although this patient had a slight degree of albuminuria (0.3 g./day), his renal function was normal and renal disease could not be blamed for his hyperkalaemia. Treatment with 9 α-fluorohydrocortisone maintained his serum potassium concentration within the range of 4.5 to 5.0 mEq./l. and, except for one episode, he was free of Stokes-Adams attacks during 1 year of follow-up.

This patient's very low levels of excretion of aldosterone suggest an inability to secrete aldosterone at a normal rate, and he also shows an inability to increase his aldosterone secretion in response to the stimulus of sodium restriction. Two abnormalities in this patient may have arisen from the absence of aldosterone, one was the excessive rise of the serum potassium concentration when on a sodium-restricted diet, the other was an increased sensitivity of the myocardium to hyperkalaemia resulting perhaps from alteration of the distribution of electrolytes between intra- and extracellular compartments in the absence of aldosterone.

A case with a different presentation of symptoms, also ascribed to

isolated hypoaldosteronism, is reported by Skanse and Hökfelt.[illegible] The patient was a housewife, aged 56, who had an 11-year history of frequent attacks of severe dizziness, weakness and easy fatiguability. She was not pigmented. Her blood pressure was 90/60 mm. Hg., but there was no postural hypotension. The only biochemical abnormality was a serum sodium concentration of 136 mEq./l. (normal range for their laboratory 141-150 mEq /l.); the serum potassium concentration was 3.5 mEq./l. Urinary excretion of 17-ketosteroids and 17-ketogenic steroids was normal and there was a brisk response to the administration of corticotrophin.

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tion to dietary restriction of potassium was abnormal; an intake of 16 mEq./day, the lowest urinary excretion attained was 36 mEq./day. Although the symptons of this patient were identical with those of patients with Conn's syndrome, analdosteronism, rather than hyperaldosteronism, was present. No cause for his hypertension was found and bilateral adrenalectomy was not followed by any change of blood pressure. *Hypokalaemia and alkalosis also persisted, as did the presence of steroid* excretion in the urine. Accessory adrenocortical tissue obviously remains in this patient.

A case is reported by Lichtwitz, Parlier, Rioco and Delaville[441] presenting with signs and symptoms typical of Addison's disease, with low resting values for 17-hydroxycorticoids and values for 17-ketosteroids within the normal range. This would appear to be a case of partial Addison's disease[1] rather than of an isolated deficiency of aldosterone secretion. No studies of aldosterone excretion were performed on this patient.

It is not yet known whether an isolated deficiency of aldosterone production is the result of defective synthesis by the adrenal cortex or of lack of trophic hormone controlling aldosterone secretion.

When urinary retention of sodium was prevented by placing the patient on a low sodium diet no attacks of paralysis could be precipitated by either the administration of 2-methyl, 9 α-*fluorohydrocortisone* or of glucose and insulin.

Analysis of skeletal-muscle biopsies obtained during the attack of paralysis showed a large excess of sodium concentration, expressed as mEq. per kg. wet weight, with a potassium concentration slightly below normal.

These interesting observations, which have not yet been confirmed, *suggest that aldosterone may play a primary role in the initiation of attacks* of paralysis in this syndrome, and that an alteration in the distribution of sodium is an integral part of the mechanism of paralysis in addition to the hypokalaemia on which attention has been focused hitherto. Conn[11] states that muscle function begins to return as soon as a sodium diuresis starts, although the serum potassium concentration may still be at its lowest level. The stimulus initiating the sudden rise of aldosterone excretion is not known, nor is it clear why aldosterone causes intracellular sequestration of potassium in this condition.

On the basis of the observations summarized above, Conn and co-workers[11] suggested that dietary sodium restriction might be of benefit in this disease as a prophylactic measure. This has been tried by de Graeff and Brocker[101] in one case, without benefit. Paralysis has also been induced in patients with this syndrome on a low sodium intake by Jones, McSwiney and Brooks,[100a] using glucose and insulin. These authors were unable to induce paralysis by the administration of DL-aldosterone acetate (2 mg.) or of 9 α-fluorohydrocortisone (1 mg.) in patients on a *sodium intake of 166 mEq./day*, and concluded that sodium retention was not essential for the development of paralysis. No relationship between the rate of excretion of aldosterone and the development of paralysis could be established by Jones and colleagues. The role of aldosterone in the pathogenesis of familial periodic paralysis awaits further elucidation.

CHAPTER XI

THE ROLE OF ALDOSTERONE IN HUMAN HOMEOSTASIS

The foregoing chapters have been essentially factual. An assessment of the role of aldosterone in homeostasis must of necessity be personal, but the data from which conclusions have been drawn will be given.

Particulars of the manner in which the existence of a sodium-retaining hormone was first suspected and of its ultimate isolation, characterization and synthesis have been outlined above. There can be no doubt about the presence of aldosterone in urine and other body fluids. Its absence from the urine of patients with Addison's disease or after bilateral adrenalectomy points to an adrenal origin, as does its synthesis by adrenal cortical tissue *in vitro* (Chapter IV). It may, however, not be the only potent sodium-retaining hormone secreted by the adrenal cortex. Deoxycorticosterone is now accepted as a secretory product of the adrenal cortex,[142, 212, 315, 636] although the quantities secreted are probably not great enough to be of physiological significance. Many substances, at present unidentified, are demonstrable on chromatograms of urine extracts and it is possible that other steroids of physiological importance to electrolyte metabolism have yet to be discovered. Sodium-retaining activity has been found very early in foetal life, from the ninth week onwards, but the substance responsible has not been identified as aldosterone.[334]

What is the role of aldosterone in human homeostasis? The amounts secreted by the adrenal cortex of a normal person under ordinary conditions of diet, exercise and temperature are such as to result in the excretion in the urine of only 5 μg. or so a day. The actual amount of this steroid secreted by the adrenal under normal conditions is not known precisely, but is almost certainly less than 500 μg. a day. Ayres and co-workers[17, 18] estimate it to be 170–190 μg. a day.

The inverse correlation between the quantity of aldosterone excreted in the urine and the amount of sodium excreted daily[61, 281, 457] points to a physiological role for aldosterone in the control of sodium excretion. However, Thorn and co-workers[457] found no significant correlation between aldosterone excretion and the sodium content of the urine at levels of aldosterone excretion below 10 μg a day. It is to be noted that a daily excretion of 1 to 10 micrograms lies within the range of normal in

normal subjects under normal conditions by the method of estimation used. Aldosterone therefore probably plays a minimal role in controlling the amount of sodium reabsorbed by the renal tubules under normal conditions of hydration and salt intake in subjects with functioning adrenal cortices. Aldosterone is reported not to be detectable in the urine when the daily sodium intake exceeds 450 mEq.[illegible]

The possibility must be borne in mind that the adrenal cortex normally secretes small quantities of a *sodium-excreting hormone*, such as that isolated by Neher, Desaulles, Vischer, Wieland and Wettstein,[illegible] which antagonizes the renal tubular activity of aldosterone. This antagonism is overcome by amounts of aldosterone equivalent to a renal excretion of 10 μg./24 hr. accounting for the observations of Thorn and colleagues.[illegible]

It is perhaps to be expected that aldosterone would be an important participant in the response of the organism to restriction of the dietary intake of sodium. It was, therefore, surprising to find[illegible] that in the majority of normal subjects placed on a low sodium intake there was a delay of 1 or 2 days before the excretion of aldosterone began to rise, although the reduction of sodium excretion by the kidneys was immediate. Administered aldosterone is excreted promptly. In the absence of data concerning secretion of aldosterone by the adrenal cortex it must be assumed that a delay in the rise of urinary excretion reflects a delayed increase of secretion. Increased 'utilization' by tissues (e.g. by the renal tubule), would be a possible explanation for the delayed rise in the rate of excretion, but there is no evidence that this occurs. Luetscher and Lieberman,[illegible] however, assert, without quoting references, that a rise of 'aldosterone' excretion occurs 'very early in the course of sodium deprivation' if bioassay methods are used for its detection

When a normal subject is submitted to a sudden reduction of sodium intake lasting for a period of days, the amount of sodium excreted in the urine falls exponentially from the first day of the reduced diet to reach a level equal to the intake by the third to fifth day. This process is accompanied by a weight loss, the major part of which occurs on the first day of the restricted intake. In the studies of Crabbé, Ross and Thorn,[illegible] maximum aldosterone excretion (measured by a physicochemical method) was achieved on the first day in only one subject of the eleven studied. In the majority of cases it was the third or fourth day, and in two cases, the sixth and last day, before maximum excretion of aldosterone was attained. In six of the eleven cases the amount of aldosterone excreted on the first day of sodium deprivation was no higher than during the control period. Again, after the rise of aldosterone excretion had reached a maximum on the third to fifth day of sodium restriction it usually fell towards normal

although during this period there was no increase in the amount of sodium excreted; this continued to be below the level of sodium intake. It follows that there is no correlation between the rate of secretion of aldosterone and the amount of sodium lost in the urine under these conditions.

Nevertheless, although aldosterone is not the agent primarily or principally responsible for initiating an increase of reabsorption of sodium by the renal tubule in response to reduction of sodium intake in the normal subject, the difference of behaviour of a patient with Addison's disease and of a patient with hypopituitarism suggests that the presence of aldosterone, even in low concentration, is necessary for the development of full efficiency in the mechanism concerned with the extraction of sodium from the glomerular filtrate, whereby the amount of sodium excreted in the urine is reduced to, or below, the amount ingested. In this respect the action of aldosterone would fall within Ingle's use of the term 'permissive', i.e. it assists tissues to adapt to the homeostatic requirements of the organism.[111] However, the interesting case reported by Skanse and Hökfelt[111] shows that the presence of aldosterone may be completely unnecessary for a normal response by the kidney to sodium deprivation in the presence of adrenal glands capable of secreting normal amounts of 17-hydroxycorticoids. The case in question, discussed on p. 99, excreted undetectable amounts of aldosterone in the urine both before and during a period of sodium withdrawal and yet the renal mechanism for sodium conservation was perfectly adequate to reduce sodium excretion to balance sodium intake within a period of 4 days. In this case, the presence of adequate (above normal) amounts of 17-hydroxycorticoid, or of some hormone as yet undiscovered, supplied whatever 'permissive' factor was necessary for adequate renal function.

A patient with hypopituitarism, on the other hand, although secreting inadequate amounts of 17-hydroxycorticoid material, still secretes aldosterone in quantities which are somewhat greater than 50% of normal. If the disease is of long standing, the patient may be unable to increase to any extent the amount of aldosterone secreted in response to sodium restriction; nevertheless this subnormal amount is sufficient to exert the necessary permissive effect and enable the renal tubules to restrict sodium excretion

In the case of a patient with Addison's disease, the failure of the adrenals to secrete both aldosterone and 17-hydroxycorticoids (or their at present unknown adrenal metabolite) results in a failure of the renal tubule to restrict sodium excretion sufficiently so as to enable sodium balance to be reached before an adrenal crisis is precipitated.

In the normal subject the role of aldosterone during a period of sodium deprivation would thus appear to be of the nature of an accessory safety mechanism. Sodium excretion is reduced exponentially towards the level of sodium intake from the first day of sodium restriction onwards by some intrinsic renal mechanism not yet elucidated. Aldosterone excretion begins to rise on the first, second or third day, varying with the subject Individual variation in the magnitude of the increase of aldosterone excretion is wide, as has been shown by Thorn and colleagues,[illegible] but is fairly constant for any given subject and bears no relationship to the efficiency with which sodium balance is achieved.

The magnitude of the aldosterone response to sodium withdrawal in the normal subject appears to correlate better with the rapidity of the weight (i.e. water) loss associated with this procedure than with any other factor, so that sudden weight loss produced by the administration of a diuretic on the first day of sodium withdrawal produces a greater rise of aldosterone excretion than does sodium withdrawal alone, although the total sodium deficit during the period of sodium withdrawal may in fact be less when a diuretic is given than when none is given.[illegible] If weight loss is prevented by the administration of vasopressin then no increase of aldosterone excretion occurs [illegible] Other experimental methods of provoking an increase of aldosterone excretion, such as vigorous exercise on a hot day, a Finnish steam bath or a phlebotomy, are also attended by weight loss. This weight loss stems from loss of extracellular fluid, hence the elevated excretion of aldosterone in these circumstances may be regarded as an attempt to defend some, as yet unspecified, compartment of extracellular fluid by stimulating the renal retention of sodium, and ultimately of water.

It can perhaps be said in summary at this stage that the secretion of aldosterone is increased in the normal subject whenever there is an acute contraction of the plasma volume or of some other monitoring body-fluid compartment. The object of this increased secretion appears to be an acceleration of the speed with which the sodium-conserving mechanism of the kidney comes into effect. The more serious and more sudden the volume deficit, the greater is the adrenal response and the more rapid and more efficient is the retention of sodium This results first in an increase of plasma osmolarity which in turn activates the production of anti-diuretic hormone leading to renal retention of water, completing the re-expansion of the plasma volume Aldosterone would appear to act with anti-diuretic hormone as a partner in a dual mechanism responsible for maintaining the volume of the vascular compartment, as outlined by Bartter[illegible] and Wolff[illegible] and their colleagues The inadequacy

of the anti-diuretic hormone system alone to fulfil this function has been commented on by Peters[illegible] and by Borst.[illegible]

If this supposition be true, namely that an increased secretion of aldosterone occurs in response to contraction of the extracellular space, then it is difficult to explain the increased aldosterone excretion in oedematous [illegible] vital area of the vascular volume which is believed to control aldosterone secretion, an argument which is not susceptible to proof until the vital volume has been defined.

A mechanism such as the following can be conceived to be acting in cases of 'secondary hyperaldosteronism', for example, in the nephrotic syndrome and in cirrhosis. Any disturbance of Starling's equilibrium[illegible] permitting an undue flow through the capillary wall of water and solutes which are not immediately returned to the vascular bed will result in a reduction of the vascular volume, stimulating the aldosterone-regulating mechanism. Although the increased aldosterone secretion results in pronounced sodium retention and eventually in fluid accumulation, the leak through the capillaries is not repaired, hence the hyperaldosteronism is perpetuated and leads to continued sodium retention and fluid accumulation. In these cases the presence of high levels of aldosterone excretion, presumably indicative of high levels of circulating aldosterone, originally designed to restore the vascular volume, no longer appears to serve a useful purpose and in fact would seem to be harmful in that it prevents the possibility of a sodium diuresis and loss of oedema or ascitic fluid. Such a situation is referred to as a 'disease of homeostasis' by Cattan and Vesin.[illegible]

Unfortunately for this simple concept, the many instances of failure to produce a sodium diuresis in patients with these oedematous states when aldosterone secretion is suppressed by the administration of amphenone B[illegible] shows that aldosterone was not a primary factor contributing to the maintenance of the oedema of these patients at the time the amphenone was given.

The position of aldosterone as part of a homeostatic mechanism concerned with the integrity of the vascular volume would appear to be a fact of the utmost importance in the formation of oedema, for without the maintenance of the vascular volume on the arterial side the continuous formation of oedema fluid could hardly be sustained.

There is increasing evidence that aldosterone, or some other secretory product of the adrenal cortex, plays a permissive role in the development

In the normal subject the role of aldosterone during a period of sodium deprivation would thus appear to be of the nature of an accessory safety mechanism. Sodium excretion is reduced exponentially towards the level of sodium intake from the first day of sodium restriction onwards by some intrinsic renal mechanism not yet elucidated. Aldosterone excretion begins to rise on the first, second or third day, varying with the subject. Individual variation in the magnitude of the increase of aldosterone excretion is wide, as has been shown by Thorn and colleagues,[111] but is fairly constant for any given subject and bears no relationship to the efficiency with which sodium balance is achieved.

The magnitude of the aldosterone response to sodium withdrawal in the normal subject appears to correlate better with the rapidity of the weight (i.e. water) loss associated with this procedure than with any other factor, so that sudden weight loss produced by the administration of a diuretic on the first day of sodium withdrawal produces a greater rise of aldosterone excretion than does sodium withdrawal alone, although the total sodium deficit during the period of sodium withdrawal may in fact be less when a diuretic is given than when none is given.[40, 41] If weight loss is prevented by the administration of vasopressin then no increase of aldosterone excretion occurs.[110, 111] Other experimental methods of provoking an increase of aldosterone excretion, such as vigorous exercise on a hot day, a Finnish steam bath or a phlebotomy, are also attended by weight loss. Thus weight loss stems from loss of extracellular fluid; hence the elevated excretion of aldosterone in these circumstances may be regarded as an attempt to defend some, as yet unspecified, compartment of extracellular fluid by stimulating the renal retention of sodium, and ultimately of water.

It can perhaps be said in summary at this stage that the secretion of aldosterone is increased in the normal subject whenever there is an acute contraction of the plasma volume or of some other monitoring body-fluid compartment. The object of this increased secretion appears to be an acceleration of the speed with which the sodium-conserving mechanism of the kidney comes into effect The more serious and more sudden the volume deficit, the greater is the adrenal response and the more rapid and more efficient is the retention of sodium. This results first in an increase of plasma osmolarity which in turn activates the production of anti-diuretic hormone leading to renal retention of water, completing the re-expansion of the plasma volume. Aldosterone would appear to act with anti-diuretic hormone as a partner in a dual mechanism responsible for maintaining the volume of the vascular compartment, as outlined by Bartter[44, 45] and Wolff[117, 133, 311] and their colleagues. The inadequacy

is given intravenously in man.[410] When aldosterone is delivered directly into the renal artery of the experimental dog,[84] a period of 60 minutes still elapses before there is any change in the excretion of sodium or potassium. The delay is the same when the substance is given into a peripheral vein of the dog. Possible explanations for the delay have been given above (p. 50).

The existence of such a lag period makes it obvious that the secretion of aldosterone does not serve as a means of rapidly altering sodium reabsorption by the renal tubule in response to transient changes in conditions of sodium, potassium or water metabolism. Indeed, it raises the question as to whether aldosterone itself has any direct effect on the renal tubule. While adrenal steroids undoubtedly modify the renal handling of sodium, possibly by an effect on the exchange of sodium for potassium and hydrion in the distal tubule,[89] there is no experimental evidence that aldosterone, or any other adrenal hormone, is the principal regulator of sodium reabsorption. As Ingle[225] has emphasized, the fact that a hormone modifies a given metabolic process does not imply that it is the principal regulator of the process. There is ample indication that factors other [illegible]

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corticosterone.[84] In some circumstances in man, e.g. Conn's syndrome, pregnancy, salt-losing nephritis and following poisoning by certain anti-oxidants,[184] the renal tubules are obviously insensitive to the presence of high concentrations of aldosterone. The ineffectiveness of aldosterone under these circumstances may be due to the simultaneous secretion of a steroid hormone with an action antagonistic to that of aldosterone. Aldosterone likewise appears to be ineffective in influencing the reabsorption of sodium during a mannitol diuresis,[408] as is deoxycorticosterone.[213a]

Generalized oedema can be produced experimentally by chronic constriction of the heart itself, of the hepatic vein or of the inferior vena cava above the junction of the hepatic vein [333] Constriction of the inferior vena cava below the function of the hepatic vein, or of the superior vena cava, does not result in the formation of generalized oedema. One common factor in the experimental conditions resulting in oedema is venous congestion of the liver. This raises the question as to whether the 'secondary hyperaldosteronism' of congestive heart failure is not in fact the result of diminished inactivation of aldosterone by the liver rather than of an increased production by the adrenal cortex Impairment of enzymic reduction of the A ring of aldosterone has indeed been noted[333b] in livers

of the oedema of congestive heart failure. If the convincing testimony in favour of the 'forward failure' hypothesis be accepted, the reduced output of the failing heart results in a greatly reduced renal blood-flow and in a lowered glomerular filtration rate. Diminution of glomerular filtration does not by itself necessarily lead to the formation of oedema, since it occurs in many conditions, e.g. Addison's disease, not associated with the accumulation of oedema. Increased tubular activity (? as a result of the presence of aldosterone) appears to be a necessary adjunct to a reduced glomerular filtration rate for the occurrence of sufficient retention of sodium to result in the accumulation of oedema. Adrenalectomy results in a sodium diuresis in dogs with experimentally produced oedema[91] and in patients with congestive heart failure[465] and ascites,[315, 116b] although it may result in still further reduction of glomerular filtration rate and additional impairment of renal function. Normal tubules respond to chronic diminution of filtered load by diminishing the reabsorption of sodium and water until balance is again attained. This they fail to do in congestive heart failure, cirrhosis and other oedematous states. Barger and co-workers[16] have shown that when hypertonic saline is injected directly into the renal artery there follows a five to tenfold increase in the excretion of sodium from that kidney, but if the same experiment is performed in a dog with experimentally produced cardiac failure the tubules reabsorb all the increased sodium load presented to them during the infusion of hypertonic saline, without change of filtration rate. Normal dogs excrete non-measurable amounts of aldosterone (as estimated by the physicochemical method of Hernando);[315] oedematous dogs with cardiac lesions excrete appreciable quantities.[466] A patient with congestive heart failure likewise fails to excrete a sodium load in a normal manner. This is not due to an inherent tubular defect since tubular reabsorption of sodium returns to normal as cardiac output increases. The increased tubular activity in cardiac failure appears to require the presence of increased amounts of circulating aldosterone for its initiation, if not for its perpetuation. The factors responsible for initially increasing the level of secretion of aldosterone in these circumstances have not yet been well delineated. Although the total circulating blood volume is increased in congestive heart failure,[419] the distribution may be unequal on the arterial and venous sides. A reduced *effective* (arterial) blood volume resulting from a low cardiac output may be the factor responsible for stimulation of the aldosterone-regulating centre.

Although the administration of aldosterone results in the increased reabsorption of sodium by the renal tubule, there is a characteristic delay of 1 or 2 hours before this effect becomes apparent, even when the compound

biological assay, found an increased excretion of aldosterone on the day of operation and on the first post-operative day, at a time when sodium retention by the kidney is intense. The sodium-retaining substance has been identified as aldosterone.[***] Venning and co-workers[****] studied three patients undergoing gastrectomy and found a raised excretion of aldosterone on the day of operation only; in only one case was there significant retention of sodium on this day. Sodium retention occurred later when aldosterone excretion had fallen to lower levels. The author[***] has noted that reduction of sodium excretion follows the pattern usually seen in the post-operative period in a patient submitted to total adrenalectomy and maintained on a constant dose of cortisone over the period of operation. The part played by aldosterone in post-operative sodium retention is by no means clear at the present time.

Aldosterone likewise cannot be blamed for toxaemia of pregnancy, since identical levels of aldosterone excretion were found in pregnant women at various stages of pregnancy, irrespective of whether toxaemia was present or not.

Although hypertension is an essential factor of Conn's syndrome, there is little evidence to implicate aldosterone in the pathogenesis of essential

In the case of an isolated insufficiency of aldosterone secretion discussed above,[***] the patient's disability (Stokes-Adams attacks) was related to an inability to maintain his serum potassium concentration within normal limits. A similar inability to control the serum potassium concentration is seen in patients with Addison's disease and more particularly in hypertensive patients who have been totally adrenalectomized and who have some degree of renal failure In this group of patients a sodium-retaining hormone such as fluorohydrocortisone must be administered, not so much to maintain the serum sodium concentration as to prevent an undue rise of the serum potassium concentration. These observations suggest that aldosterone plays an important role in the maintenance of the serum potassium concentration. This may be in part through its action on the renal tubules, in part possibly through a direct action in controlling the permeability of cell membranes to the potassium ion.

It is apparent from the above that the physiological role of aldosterone in man is as yet ill defined. There are indications that it plays little part in regulating sodium excretion under normal conditions of hydration and

from rats with inferior vena cava constriction. A diminished rate of inactivation of aldosterone by the congested liver, although it may occur, is not a major factor contributing to the increased rate of excretion of aldosterone by the kidney in cases of congestive heart failure, since an increased concentration of aldosterone in adrenal venous blood has been found by Davis and colleagues[illegible] in dogs with vena caval obstruction The importance of aldosterone in the production of congestive heart failure, oedema and ascites is again emphasized by other observations of Davis and colleagues,[illegible] namely that constriction of the inferior vena cava alone did not lead to the production of ascites; the addition of salt-retaining hormone, in this case deoxycorticosterone, resulted in ascites production.

Previous observations by Davis and colleagues[illegible] further confuse an understanding of the sodium retention occurring during congestive heart failure, since they found that sodium balance could be maintained in adrenalectomized dogs by the administration of 3 mg of deoxycorticosterone acetate and 25 mg. of cortisone. Following the production of congestive heart failure by partial occlusion of the pulmonary artery, the dogs retained sodium when only one milligram of deoxycorticosterone acetate (plus 25 mg. cortisone) was given daily, i.e. the requirement of sodium-retaining hormone was reduced. Withdrawal of the sodium-retaining hormone resulted in a negative sodium balance. This experi-

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plays a part in the initiation of sodium restriction in congestive heart failure. Vander, Malvin, Wilde and Sullivan[illegible] believe this to be an increased filtration fraction.

The inference that can be drawn from these numerous studies of the sodium retention and oedema formation occurring in congestive heart failure, the nephrotic syndrome, cirrhosis and other states is that aldosterone is one, but not the only, factor necessary for the initiation, if not for the continuance, of retention of sodium by the renal tubules; that the stimulus is a change in pressure or blood flow in the right atrium of the heart or in the arterial circulation, and that the purpose of the state of hyperaldosteronism is to defend the integrity of the plasma volume in some unspecified, but vital, area of the circulation.

The limited response of aldosterone excretion to the administration of corticotrophin in man suggests that aldosterone is not responsible for the sodium retention that accompanies corticotrophin therapy.

Aldosterone has been held responsible for the sodium retention that follows surgical operations.[illegible] Llaurado[illegible] using a method of

biological assay, found an increased excretion of aldosterone on the day of operation and on the first post-operative day, at a time when sodium retention by the kidney is intense. The sodium-retaining substance has been identified as aldosterone.[illegible] Venning and co-workers[illegible] studied three patients undergoing gastrectomy and found a raised excretion of aldosterone on the day of operation only; in only one case was there significant retention of sodium on this day. Sodium retention occurred later when aldosterone excretion had fallen to lower levels. The author[illegible] has noted that reduction of sodium excretion follows the pattern usually seen in the post-operative period in a patient submitted to total adrenalectomy and maintained on a constant dose of cortisone over the period of operation. The part played by aldosterone in post-operative sodium retention is by no means clear at the present time.

Aldosterone likewise cannot be blamed for toxaemia of pregnancy, since identical levels of aldosterone excretion were found in pregnant women at various stages of pregnancy, irrespective of whether toxaemia was present or not.

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will result in an increase in aldosterone secretion.

In the case of an isolated insufficiency of aldosterone secretion discussed above,[illegible] the patient's disability (Stokes-Adams attacks) was related to an inability to maintain his serum potassium concentration within normal limits. A similar inability to control the serum potassium concentration is seen in patients with Addison's disease and more particularly in hypertensive patients who have been totally adrenalectomized and who have some degree of renal failure. In this group of patients a sodium-retaining hormone such as fluorohydrocortisone must be administered, not so much to maintain the serum sodium concentration as to prevent an undue rise of the serum potassium concentration. These observations suggest that aldosterone plays an important role in the maintenance of the serum potassium concentration. This may be in part through its action on the renal tubules, in part possibly through a direct action in controlling the permeability of cell membranes to the potassium ion.

It is apparent from the above that the physiological role of aldosterone in man is as yet ill defined. There are indications that it plays little part in regulating sodium excretion under normal conditions of hydration and

electrolyte intake, but circumstances resulting in contraction of circulating effective blood volume may lead to increased secretion of aldosterone and so to sodium retention. The sequence of events is possibly as follows:

Contraction of effective blood volume, whether this be on the arterial or venous side is not clear at the present time, influences a receptor situated outside the cranium, situated either in the right atrium or somewhere on the arterial circulation. Afferent fibres from this receptor influence a centre situated in the mesencephalon or diencephalon. Stimulation of this centre releases a trophic hormone, aldosterone-stimulating hormone, which in turn promotes the synthesis and release of aldosterone into the adrenal vein, resulting in alteration of renal tubular activity. Sodium loss in the urine becomes minimal, and, if the sodium intake is adequate, total body sodium will increase and serum osmolarity will rise. The secretion of anti-diuretic hormone will in turn be increased, resulting in decreased renal free-water clearance and retention of water. This leads to expansion of the plasma volume, tending to restore it towards normal. If the primary pathological condition present is associated with an abnormality of fluid exchange across the capillary wall, as in the nephrotic syndrome or in cirrhosis, the circulating blood volume is never restored to normal and a state of hyperaldosteronism is perpetuated. One of the difficulties in explaining the persistent hyperaldosteronism of the nephrotic syndrome or of cirrhosis is that the total plasma volume in these conditions is either normal or above normal. However, if in these conditions the primary pathological condition, a low colloid osmotic pressure, leads to an excessive loss of fluid from the circulation at capillary level, the arterial blood volume, which cannot be measured, may in fact be low. Persistence of a low arterial blood volume might account for a continued excessive secretion of aldosterone.

Increased excretion of aldosterone results from the administration of diuretics or restriction of sodium intake. If it were certain that the sodium retention seen in oedematous patients was due solely to increased circulating aldosterone it would be illogical to treat oedematous patients with diuretics and a low salt diet. It would be more logical to use agents which suppress aldosterone secretion or antagonize its action on the kidney. Experience with the administration of amphenone, however, suggests that in many cases aldosterone is not a major factor responsible for the sodium retention found in these cases.[illegible] Success has been claimed for the treatment of patients with cirrhosis, nephrosis and congestive heart failure by the administration of prednisone,[illegible] but it is not clear whether the reduction of aldosterone excretion precedes or follows the sodium diuresis.

The role of aldosterone in the causation of Conn's syndrome is likewise by no means clear. Defining this syndrome as the association of hypertension with hypokalaemia, it is clear that an excessive secretion of aldosterone is not its sole cause, since many typical cases have now been reported in which the excretion of aldosterone in the urine was normal. Even in the cases in which an excessive excretion of aldosterone in the urine has been alleged, positive chemical identification of the responsible substance as aldosterone has been made in very few. It can probably be produced by the excess production not only of aldosterone but of a number of steroid hormones, of which corticosterone is one, either alone or acting in association with normal quantities of aldosterone. It differs in this respect from secondary aldosteronism, in which there is excessive excretion of aldosterone with normal production of other corticosteroids. This may account for the existence of excessive renal loss of potassium in primary aldosteronism and not in secondary aldosteronism. The syndrome is of importance in that it is another form of hypertension amenable to surgical treatment, with restoration of the blood pressure to normal providing irreversible anatomical damage has not been sustained by the kidneys.

There remains for discussion the mode of action of aldosterone. Its site of action is almost certainly the cell membrane. It would appear that its principal function is to alter the 'permeability' of the membrane to sodium and potassium. To effect this, it can be postulated that it acts, as other hormones seem to act, by influencing the rates of enzyme-catalysed reactions. A cell membrane is a lipoprotein structure in which are discontinuities ('pores'), in a gradation of sizes, which will permit the passage of water-soluble molecules or ions of dimensions smaller than that of the pore diameter. Lipoid soluble substances can enter the cell by solution in the cell membrane. Water soluble, lipoid-insoluble substances whose molecular diameter is greater than the pore diameter will obviously experience difficulty in passing through the cell membrane. The sodium ion, in its hydrated form, may fall into this category If the ion can be rendered more lipoid soluble, permeation across the cell membrane would be accelerated. Sulser and Wilbrandt,[478a, 524a] noting that aldosterone antagonized the inhibitory action of cardiac glycosides on sodium and potassium flux across the red cell envelope, have suggested that it acts as a chelating agent, binding sodium and potassium ions. This, by rendering the complex more lipoid soluble than the uncomplexed ion, would facilitate its transfer into and out of cells.

The cell membrane, however, even in the presence of aldosterone, cannot be freely permeable to sodium and potassium since a very con-

siderable concentration gradient of both sodium and potassium exists across the cell membrane, the maintenance of which must involve the expenditure of energy. The mechanism whereby this gradient is achieved is imperfectly understood; current thinking favours the presence of a 'sodium pump', a carrier system which actively transfers sodium from intracellular to extracellular fluids. Aldosterone may play some part in this system. Unlike the glucocorticoids, aldosterone in physiological amounts has no effect on the distribution of water between intra- and extra-cellular compartments. The chief effect of aldosterone is on the renal tubular regulation of sodium reabsorption and perhaps also on the active secretion of potassium and hydrion. Its importance in the regulation of sodium and potassium transfer in other tissues has yet to be elucidated.

Aldosterone remains one of the most elusive of hormones. Its existence, long suspected, has been proved and its constitution elucidated, but delineation of its exact role in homoestasis and of its importance in clinical medicine now awaits the development of more satisfactory analytical procedures.

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